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Historical Land Fragmentation and its Effects on Genetic Diversity and Parasitism of Island Populations of Podarcis erhardii (Lacertidae, Reptilia)

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ABSTRACT

The Pleistocene land bridge islands in the Aegean Sea make an ideal natural experimental system for testing how island age, area and isolation affect genetic variation. My research focuses on the population genetics of the Aegean wall lizard *Podarcis erhardii* (Lacertidae, Reptilia), which because of its wide distribution, and poor dispersal abilities is a sensitive indicator of fragmentation history. I predict that genetic diversity will be positively correlated with island area and negatively correlated with age. I also predict that island characteristics, host genetic variability or grazing may impact parasite prevalence. Findings showed that larger islands maintained more genetic diversity than smaller islands and older islands have higher mite loads. Geographic distance was unrelated to genetic divergence. Tick prevalence was significantly associated with past grazing practices. This study provides a unique opportunity to disentangle factors that may influence the retention of genetic diversity and prevalence of ectoparasites in natural populations.

INTRODUCTION

This study combines knowledge of the history of fragmentation with patterns of population genetic variation to determine which island characteristics have the greatest impact on the retention of genetic diversity in Erhard's wall lizard, *Podarcis erhardii,* a small lizard ubiquitously distributed throughout the Aegean Sea. The aims of this study are threefold: First, I use mitochondrial sequence variation to examine the phylogeographic history of island populations. These data were used to identify which islands share a common fragmentation history. The second aim of this study is to use non-coding hyper-variable nuclear markers to examine how island age, size and distance to the nearest landmass affect lizard population genetic diversity. Third, this study examines the relationship between genetic diversity, grazing history and ectoparasite prevalence to understand what historical and ecological factors may influence host ectoparasite loads.

Island biogeography theory

Mac-Arthur and Wilson's model of island biogeography (1963) predicts that the number of species present on a given island will reach a dynamic equilibrium based upon rates of extinction and colonization. Island biogeography theory also predicts that large islands with greater areas support larger populations and islands closer to the mainland have more colonization opportunities and therefore more immigrants, resulting in higher species equilibrium (Mac-Arthur & Wilson 1963). Conversely, small populations are highly susceptible to drift and are therefore likely to lose genetic variability and face inbreeding depression. Consequently, it has been argued that island populations, because they are smaller than their mainland counterparts, have lower levels of genetic variation and are more vulnerable to extinction (Frankham 1995, 1998). By understanding the predictions of island biogeography we can use these same principles to make predictions about how factors such as age, area and distance from mainland will affect measures of genetic diversity.

Several studies have applied MacArthur and Wilson's predictions for species richness in the Aegean archipelago (Dillon and Wethington, 1995; Fattorini 2002; Bittkau & Comes 2005). Fattorini (2002) examined 32 islands and 166 taxa of tenebrionid (Coleoptera, Tenebrionidae) beetles to determine which eco-geographical (latitude, longitude, area, distance to mainland and distance to nearest landmass) variables had the greatest impact on species richness. Although area accounted for most of the variability in the number of species found on each island, they also noted a distinct decline in the number of Balkan taxa from west to east, while Anatolian (Asia Minor) taxa showed the opposite trend. To explain these

findings, the authors postulated that the islands of Aegean were colonized via a series of land bridges with the Anatolian taxa colonizing from what is now known as Crete, while the Balkan taxa descending from the mainland of Greece. Consequently, the species present on the islands today represent fauna from a mix of these two different sources.

While the theory of island biogeography has been used to predict equilibrium species number in continental land fragments (Case 1975; Wilcox 1978) this theory has rarely been extended to population genetic data. A theoretical study by Johnson et al. (2000) argued that larger and more distant islands are more divergent because they are more resistant to the influx of immigrant (divergent) alleles compared to smaller, more distant islands. Area is also thought to be more important than distance in retaining both divergence and polymorphism because the distance effect will eventually saturate (i.e. that there is a distance threshold, beyond which immigration ceases to be important) whereas the probability that colonist alleles will persist increases with island area. In contrast, the Aegean study system presented here is believed to be largely extinction driven with little to no gene flow between islands (Beerli 1996). Therefore, in our system, where immigration is thought to be absent, initial allelic richness will only be maintained on large islands, while small islands will lose alleles as loci drift to fixation (prediction 1). I also further predict that if immigration is absent, then genetic diversity will decline with island age due to the cumulative effects of genetic drift (prediction 2) and because of the lack of gene flow between islands there will be no relationship between geographic and genetic distance (prediction 3). We will test these predictions using a series of Aegean land fragment islands known as the Cyclades.

The effects of bottlenecks on genetic diversity

Population bottlenecks have been widely studied by evolutionary biologists to determine how dramatic reductions in population size affect genetic diversity (Wright 1931; Nei et al. 1975). More recently, understanding the effects of population bottlenecks on genetic variation and also population viability has become increasingly important to conservation biologists and population geneticists (Cornuet & Luikart 1996; Luikart & Cornuet 1998). Passing through a population bottleneck can adversely affect threatened species in many ways including a loss of genetic variation (Lande 1988), increased inbreeding, increased susceptibility to stochastic processes and increased likelihood of extinction (Frankel & Soule 1981; Lande 1994; Garza & Williamson 2001). Since each of the island populations studied here conceivably experienced a population bottleneck at island inception, understanding the impacts of such an event on levels of inbreeding, allelic variation and overall heterozygozity is highly pertinent to our study system.

Population bottlenecks result from a dramatic decrease in population size, whereby original heterozygozity is lost as a function of population size ($H_O/H_t = 1 - 1/2N$) where H_O= initial heterozygozity at time 0, H_t= heterozygozity at time t and N= the number of individuals (Crow & Kimura 1970). However, heterozygozity can subsequently be regained through population growth (Nei et al. 1975). In contrast, allelic richness has been shown to be a more sensitive indicator of bottleneck effects than heterozygozity, in both empirical (eg. Leberg 1992) and theoretical studies (eg. Luikart et al., 1998). This is because rare alleles are more likely to be lost following a population bottleneck, thus resulting in a distortion of pre-bottleneck allele frequencies and loss in allele number.

There are numerous approaches for determining the genetic signatures of past population bottlenecks (Garza & Williamson 2001; Spencer et al. 2000). These tests of bottleneck effects include: distortion of allele frequency distributions (Luikart et al., 1998), the mean ratio of the number of alleles to the range in allele size, where the statistic M is equal to k/r, and k is the number of alleles and r the range in allele size (Garza & Williamson, 2001), heterozygozity excess with respect to mutation-drift equilibrium (Cornuet & Luikart, 1996), and temporal changes in allele frequencies (Tajima & Nei 1984; Waples 1989). Both latter measures have been shown to be reliable indicators of recent bottleneck events (Spencer et al., 2000; Beebee & Rowe, 2001). Unfortunately, heterozygote excess persists for only for a few (0.2-4.0 N_e) generations (Luikart & Cornuet 1998) before reaching a new equilibrium between mutation and drift, making this test unsuitable for long-term population bottlenecks. Also, temporal shifts in allele frequencies require two sampling periods making neither measure suitable for our study system. However, the progressive decline in heterozygozity and loss in allelic richness in the absence of population growth and/or immigration should be readily detectable in our islands. Furthermore, distortion in allele frequencies and M, the mean ratio of alleles to the range in allele sizes, should also be discernable in our populations. Perhaps of greater importance to the present study is determining how the severity and duration of past bottleneck events has impacted genetic diversity. In this regard, the Aegean islands are well suited to answering these questions and provide us with a unique opportunity to tease apart both the effect of the magnitude (area) and duration (time since isolation) of the bottleneck on different measures of genetic variation.

Many studies have demonstrated that population bottlenecks result in inbreeding and loss of genetic diversity (eg. O'Brien et al. 1985, 1987). This finding brings up several questions regarding the potential fitness costs associated with population bottlenecks. Because population bottlenecks are often an inevitable part of captive breeding program, conservation biologists are particularly interested in

determining to what extent a loss in genetic diversity may compromise fitness. It has been postulated that a loss in genetic diversity may result in a reduction in immuno-competence and increased susceptibility to parasite infection, both of which could have an associated fitness cost. However few studies have examined the effects of bottleneck history on these variables (Coltman et al. 1999; Reid et al., 2003; Hawley 2007; Hale & Briskie 2007). Researchers have also questioned whether or not a fitness cost is associated with a loss in genetic diversity, noting that no studies have shown that a wild population has declined due to a lack in genetic diversity (Lande 1988). It has also been argued that there is no evidence that a reduction in immune response following a bottleneck is anything more than a transient effect (Tompkins 2007) or that immuno-competence (as it is currently measured) is an adequate measure of fitness (Caro & Laurenson 1994; Thompkins 2007; Hawley 2007; Smits 2007) due to seasonal and breeding condition differences in immune function (Tompkins 2007). Others argue that inbreeding increases a population's likelihood of extinction and susceptibility to stochastic processes, so that regardless of the exact cause of extinction, genetics plays an important role in effective management of captive populations and should be monitored accordingly (Caro & Laurenson 1994). In some cases, inbreeding can be correlated with fitness (Frankham 1995; Mackintosh & Briskie, 2005), with inbred individuals suffering from inbreeding depression, manifested in a reduction in their ability to mount an effective immune response (Frankham 1995; Reid et al. 2003) and a decrease in offspring production (O'Brien et al. 1985).

A recent study conducted by Hale and Briskie (2007) addressed how population bottlenecks, and the inbreeding experienced by the population, affects an individual's immune response and parasite load. While they were able to demonstrate a reduction in immuno-competence using phytohaemagglutinin (PHA) assays in the inbred population (founder population n=5), it was only significant in one season. Additionally, they found that there was no significant difference in ectoparasite loads between the severely bottlenecked population and an outbred source population. This runs contrary to their predictions and suggests that their choice of parasites were either inappropriate or that they overlooked several other complicating factors that could confound interpretation of their analyses. For example, one class of ectoparasites examined were feather mites, which have been shown previously to be commensal organisms and are therefore not likely not trigger an immune response or be affected by host inbreeding levels (Smits 2007).

Another major drawback to Hale & Briskie's 2007 study was that it was a two sample comparison (Hawley 2007), thus greatly reducing the power to detect a meaningful effect. In contrast, the present study provides an unrivaled opportunity for examining the effects of bottleneck events on both

immuno-competence and parasite burden, because each island population conceivably passed though a bottleneck event where both the severity (age) and magnitude (area) can be determined.

The Aegean as a model system

The Aegean is comprised of islands of different sizes, ages and isolation histories, thus providing an ideal opportunity to test the effects of habitat diversity, island area and distance between islands and mainland (Foufopoulos & Ives 1999; Fattorini 2002; Bittkau & Comes 2005). Several studies have directly examined the loss of genetic diversity on small island populations compared to mainland counterparts (for review see Frankham 1997). However, the effects of specific island characteristics on population genetic diversity have received relatively little attention.

A study conducted by Seddon and Baverstock (1999) examined the loss of genetic variation in island populations of the Australian bush rat using the major histocompatibility complex (MHC). They noted a substantial lack of MHC variation in island populations compared to mainland counterparts. They suggest that this was due to random genetic drift on the islands. Another study conducted on the black-footed rock-wallaby (Eldridge et al., 1999) compared island and mainland populations to assess the effect of small population size and isolation on genetic variation. Results from this study showed that island populations were highly inbred and had exceptionally low levels of genetic diversity. Capula and Ceccarelli (2003) found the same trend in the Mediterranean lizard *Podarcis sicula*, with island populations being less genetically diverse than their mainland counterparts.

To date, many studies on the genetic effects of habitat fragmentation have noted that as habitats become increasingly fragmented, the population size declines, inbreeding increases and genetic variability decreases (Galeuchet et al. 2005, Hooftman et al. 2004). Montane sky islands provide an excellent opportunity to examine how diminishing islands of suitable habitat can affect richness and genetic diversity. For example, Knowles and Richards (2005) found that the genetic divergence observed in montane grasshoppers was the result of past range fragmentation and the subsequent repartitioning and loss of ancestral genetic variability.

Understanding how fragmentation history affects genetic diversity is an important problem in conservation biology and the islands of the Aegean represents an ideal system for testing basic predictions of area, age and isolation on genetic variability. Islands in the Aegean Sea are comprised almost entirely of continental land fragments that were created as a result of rising sea levels since the last glacial

maximum (Van Andel & Shakleton 1982). As detailed bathymetric data is available, the impact of time since isolation and the magnitude and duration of the bottleneck that resulted from this isolation can be examined. Assuming island area is a reliable indicator of reptile population size, this study system therefore provides a unique opportunity to directly test the effects of demographic history on the genetic variability in a naturally replicated system.

Continental land fragments in particular provide an excellent opportunity to model the effects of historical isolation, and genetic drift on populations (Bittkau & Comes 2005; Gillespie & Roderick 2002). This is because fragment islands offer not only a series of naturally replicated isolation events, but also, in instances where immigration is likely to be unimportant, are dominated by drift (Capula 1996; Galeuchet et al. 2005; Floyd et al. 2005), thus greatly simplifying the interpretation of their fragmentation history. Brown (1971) suggests that a non-equilibrium model be applied to fragment islands (mountain tops as well as continental shelf islands), where extinction determines the number of species present on a given island instead of immigration. This model could easily be extended from species number to the number of alleles expected to persist on an island without immigration.

The Aegean Archipelago is characterized by both a complex paleogeographical history (Anastasakis & Dermitzakis 1990; Hausdorf and Hennig 2005; Lambeck 1996), and high levels of morphological variation, genetic diversity and endemism in reptiles, birds, plants and invertebrates (Fattorini, 2002; Strid 1970; Sfenthourakis, 1996, Sfenthourakis & Legakis, 2001; Chatzimanolis, 2003). Interestingly, a study by Bittkau and Comes (2005), which examined chloroplast DNA variation of *Nigella arvensis* (Ranunculaceae) distributed throughout the Aegean Sea, found three distinct lineages, which they postulate resulted from past fragmentation of a once pan-Aegean population. They hypothesized that fragmentation was due to post-Messinian sea flooding, followed by Pleistocene eustatic shifts, which together determined the pattern of island formation observed today. Using molecular data they addressed two predictions regarding the Cyclades: (1) population subdivision (genetic drift and restricted gene flow) would be greater on islands than on the mainland, and (2) no pattern of isolation by distance would be apparent due to random genetic drift following fragmentation. Consistent with their first prediction, they observed a greater amount of population subdivision on islands than on the mainland. Island populations also had lower haplotype diversity and allelic richness compared to the mainland. Many (60%) of the sampled sites were fixed for one haplotype, and in the case where there was more than one haplotype, there were few variants. In keeping with their second prediction, they found no correlation and therefore concluded that there was essentially no gene flow.

Beerli et al (1996) combined molecular data with well studied geological/historical events in the Aegean (12,000 ya, 1.8 mya, 2-3 mya, and 5.2 mya) to calibrate a protein-based molecular clock in five Aegean water frog species. The authors surveyed genetic variation in 33 pairs of neighboring populations of frog populations using 31 electrophoretic loci and found a linear relationship between predicted geologically determined separation (age) and genetic distance (Hudson 1982). Genetic data also showed that island populations had fewer alleles per locus, and lower heterozygozity than mainland populations. The authors offered three possible explanations for their results: (1) vicariance (2) introduction by humans (3) over-water colonization. In the case of vicariance, they postulated that a widely distributed species became subdivided due to sea level changes resulting in the isolation of populations on newly formed islands. The subsequent bottleneck these populations experienced distorted allele frequencies, causing them to drift to fixation. In the case of introduction by humans, they suggest that a few frogs may have been brought by humans, (either on purpose or by accident) to an island where a new breeding colony was established, resulting in reduced genetic diversity due to founder effect. However, the third explanation of over-water dispersal does not seem plausible as frogs absorb both water and salt through their skin thus making salt water an impermeable barrier to dispersal.

Capula and Ceccarelli (2003) examined erosion of alleles and genetic variability in mainland and insular populations of the related species *P. sicula*. Using allozymes they measured population genetic diversity and calculated F-statistics to estimate genetic distance between islands. Capula and Ceccarelli (2003) found that a high percentage (60%) of scored loci were monomorphic in all individuals sampled from the same locality. Of the polymorphic loci examined, they found that there was a significant level of heterogeneity suggesting localized differentiation. Overall they observed the highest amount of genetic diversity (measured as heterozygozity) in southern Italy, with central Italy having some of the lowest levels of genetic diversity. In contrast, insular populations appeared to be relatively genetically depauperate. The low level of genetic diversity observed in island populations could be due to either founder effects or genetic bottlenecks. The authors argue for founder effects based on the fact that island populations have no unique alleles, most alleles are fixed at each locus, and they are very similar to populations from central Italy. They suggest that a small number of individuals were unintentionally introduced from central Italy in historical times. Not surprisingly, *P. sicula* has proven to be a most prolific colonizer, and has been shown to readily adapt to different environments following long-range introductions with naturalized populations found in Spain, France, Portugal, Turkey, South Africa, and the USA (Capula and Ceccarelli 2003). *P. erhardii*, on the other hand, has not been found outside of its expected range and has shown a very limited amount of long distance dispersal (Valakos et al. 1999).

In contrast to these preceding studies, the study presented here uses both (a) mitochondrial cytochrome b sequence data to reconstruct the biogeographical history of the Cyclades and (b) highly variable microsatellites to examine how island history affects genetic diversity at the population level. This combination of markers provides a more complete picture of what factors are important in governing the retention of allelic diversity on land fragment islands where the isolation history of each island is known. This study also adds substantially to previous phylogeographic studies (Poulakakis et al., 2003, 2005) in that much of this work has been carried out using large population-level sample sizes instead of only a few individuals from each island.

Reconstruction of island history in the Aegean

Knowing both the isolation history and the age since island formation provides us with a powerful tool for examining the effects of island history, area and age on genetic diversity. It has been widely accepted that sea levels were 120 meters lower than they are now during the last glacial maxima (LGM) 18,000-20,000 years ago (Lambeck 1996; Lambeck & Chappell 2001; Van Andel & Shakleton 1982). Consequently, what now exists as a complex of islands was at one time a single contiguous land mass referred to here as the protocyclodic block. Because the rate at which sea levels have risen since the LGM is known for the islands of interest, it is then possible to determine the sequence and time of island isolation.

To determine the sequence of fragmentation, three things must be known about the area of interest. First, detailed bathymetric data must be obtained; second, the geologic and geographic history of the study area must be stable; and lastly any change in observed sea level due to eustatic effects must also be known (Lambeck 1996). Eustatic sea level changes are important to consider because sea level changes are not uniform across the entire globe (Lambeck, 1996). The Aegean Sea has been traveled for thousands of years by humans and a detailed bathymetric survey is available. Bathymetric surveys are deduced from detailed coastal geometry and shallow water isobaths and are used primarily for maritime navigation. Additionally, geologists have studied the region extensively and have a working knowledge of the geology of the Aegean Sea (Van Andel & Shackleton 1982; Lambeck 1996). Finally, the amount of land uplift and subduction due to seismic effects has been quantified using archeological sites in conjunction with carbon and oxygen isotope dating (Lambeck 1996). These studies have enabled the reconstruction of the sequence of island formation and inference of the time of isolation (for reviews see Van Andel & Shackleton 1982; Shakleton 1984; Lambeck 1996) allowing fragmentation history to be inferred. The time that isolation occurred can also be extrapolated using sea level curves for the region

taking into account eustatic sea level changes (Figure 1). A sea level curve is based on several factors: eustatic sea-level changes, the hydro-isostatic contribution from the melting of glaciers and changes in sea levels due to tectonic activity (Lambeck 1996). Sea level curves are represented using a two-dimensional graphic with time on the x-axis and the change in sea level on the y-axis (Figure 1).

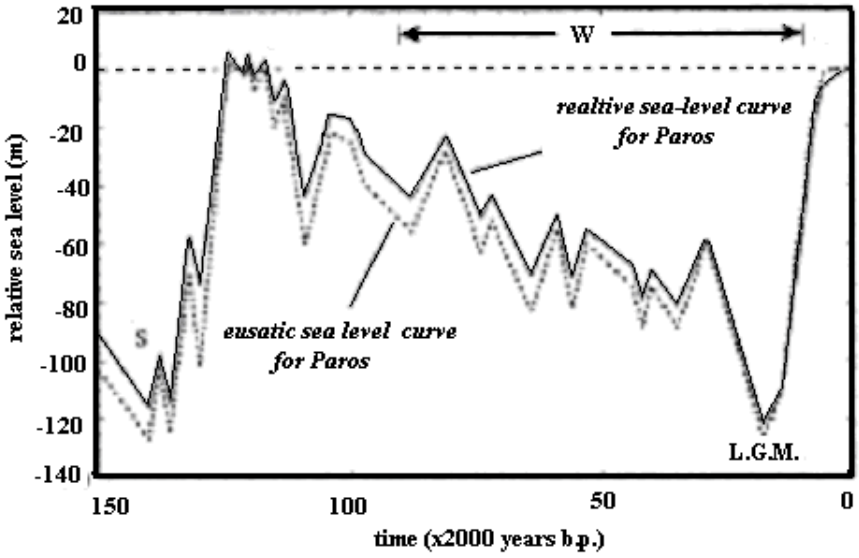

Figure 1

Relative sea level curve for Paros is depicted with a solid line. Eustatic sea level fluctuations are depicted with a dashed line. Figure modified from Lambeck

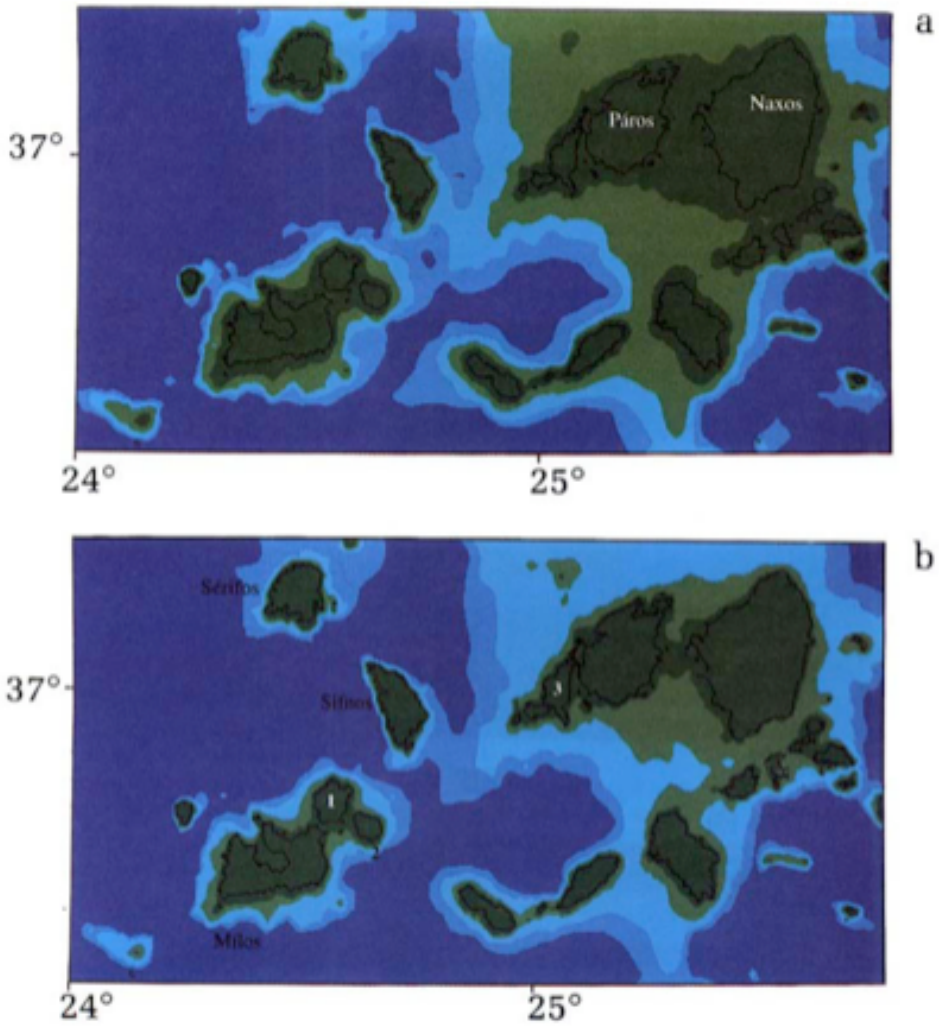

Figure 2: Land fragmentation of the Cyclades (Taken from Lambeck, 1996). Present day island areas are shown in black.

a. Depicts the geography of the region 18,000 years ago
b. Depicts the geography of the region 12,000 years ago

Our study focuses on the following islands: Agrilou (Ag), Andreas (An), Antikeros (Ak), Glaronissi (Gl), Iraklia (Ir), Keros (Ke), Kopries (Kp), Koufonissi (Ko), Makronissi (Mk), Megalos Ambula (Ma), Magali Plaka (Mp), Naxos (Nx), Nea Kameni (Nk), Ovriokastro (Ov), Phtira (Fi), Santorini (Sa) and Schoinoussa (Sk). These islands are distributed throughout the central Aegean Sea, North of Santorini and west of Amorgos (Figure 3).

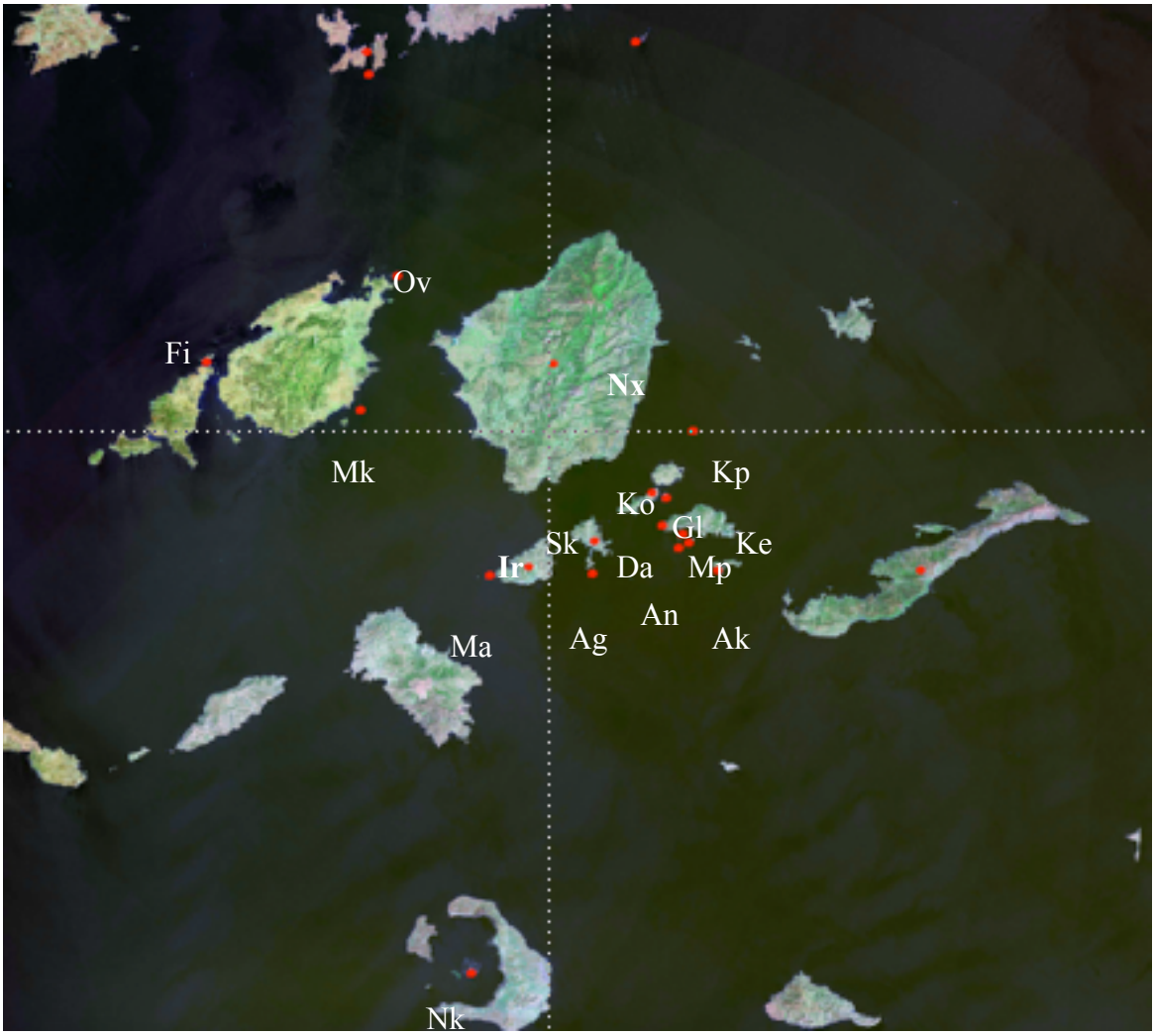

Figure 3: Map of Cyclades with islands sampled marked with red dots. Those where genetic analyses were carried out are shown with a two letter code in white. Table 2 provides the letter code for each island.

Study organism

Due *to P. erhardii*'s widespread distribution in the Aegean Sea, its poor over-water dispersal abilities (Johannes & Ives 1999), and relative ease of capture, we chose this species as a model to examine the effects of island history on population genetic variation. The Aegean wall lizard *P. erhardii* was first described in Bedriaga in 1876. The range of *P. erhardii* consists of the southeastern portion of mainland Greece and extends into the Western Aegean islands (Figure 4).

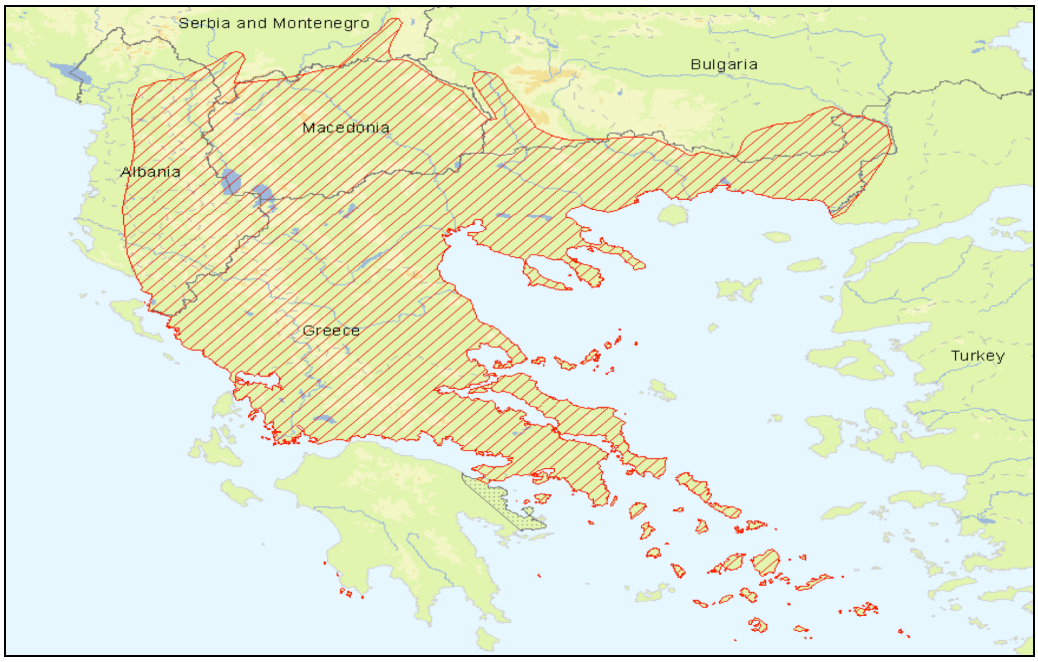

Figure 4: Red indicates the current range of *Podarcis erhardii.*

Podarcis erhardii is usually between 60-70 mm snout-vent-length (SVL) with a tail twice as long as the body (Arnold & Ovenden 2002). They are sexually dimorphic, breed seasonally and lay one clutch per year. Their diet consists primarily of small snails and arthropods (Valakos 1986). This small lizard is a habitat generalist preferring open rocky habitat with sparse vegetation and ample basking opportunities, and occurs in high densities across the open *maquis* habitat of the study islands (Gruber 1986; Valakos 1986).

A study conducted in the Aegean (Foufopoulos and Ives 1999) examining how life history traits of Aegean reptiles affected their vulnerability to extinction found that *P. erhardii* is one of the least extinction prone reptile species. Extinction rates were estimated by either assuming all islands fragmented simultaneously or by taking the historical sequence of fragmentation events into account. Specifically the study explored how traits such as body mass, longevity, abundance and habitat specialization affected species vulnerability to extinction. They found that the best fitting model (with or without sequential isolation) identified both reptile abundance and habitat specialization as significant factors in influencing a species' persistence. The higher the habitat specificity and the lower the abundance the more vulnerable a species is to extinction. This is a particularly interesting finding when we consider the life history traits of *P. erhardii*. From Appendix A in Foufopoulos and Ives (1999), we see that the low habitat specificity for *P. erhardii* (8 out of a possible 9 habitats found throughout the Aegean are exploited) and its high abundance value (4 out of a possible 4) indicate that it has a very high likelihood of persisting on islands.

Indeed, the fact this it is one of the least extinction prone species examined underlies the utility of *P. erhardii* as a model species for the present study.

Previous studies using 400bp of mitochondrial DNA demonstrate that there are two distinct lineages of *P. erhardii* (Poulakakis et al., 2003). The first, or the western clade, occurs in Crete and on its associated islets (*P. e. lekaorii, P. e. cretensis, P. e. schiebeli, P. e. punctigulris, P. e. elephonisii, P. e. rechingeri* and *P. e. wernerianus*). The second occurs in the Cyclades, the Sporades and Dodekanesa islands and their associated islets (*P.e. naxensis, P.e. ruthveni, P.e. amorgensis, P.e.andreas, P.e.syrinae*). Their study also showed that *P. erhardii* is paraphyletic with *Podarcis peloponnesiaca* clustering between *P. erhardii* lineages derived from Crete and Pori (Poulakakis 2003). The present study focuses on islands within the second clade, and samples both new and previously sampled islands. In addition to understanding the effects of island history on genetic diversity, the current study hopes to provide a more resolved phylogeny of this clade of *P. erhardii* and in so doing provide novel insights into the biogeographical history of the Aegean.

Genetic Markers

Mitochondrial sequence data provides a phylogenetic framework with which to group island populations into historically isolated units and when used in conjunction with bathymetric data can be used to infer fragmentation history. Mitochondrial markers are often the marker of choice for inferring phylogenies (Moore, 1995) because the mitochondrion is non-recombining, has relatively high levels of variability (Jarne & Lagoda 1996) and is sensitive to drift because of its smaller effective population size (one fourth that of nuclear markers). These properties make it ideal for fine-scale resolution of historically isolated lineages. Mitochondrial markers are not without their drawbacks, however. In addition to being maternally transmitted, the mitochondrion may potentially be under selection (Bazin et al. 2006) and recombination (Tsaousis et al., 2005). Additionally nuclear translocations of mitochondrial DNA have been detected previously in *P. sicula* (Podnar et al. submitted 2006). Because of this, nuclear and mitochondrial data must be carefully examined for any evidence of frame shifts or indels within coding sequence in order to determine the presence or absence of these pseudogenes.

In keeping with earlier work on *P. erhardii* (Poulakakis et al., 2003) a 430bp fragment of the cytochrome b gene was chosen for the present study. Cytochrome b has been shown previously to be more variable than the control region (Brehm et al. 2002) and therefore was chosen as the mitochondrial marker for this study. Although cytochrome b data is unlikely to be variable enough for us to resolve

effects of history on genetic diversity, it nevertheless allowed us to select island groups that share a common fragmentation history and identify fragments derived from the same "mother" island during the last glacial maximum (LGM) ~18,000 years ago.

Many studies have highlighted the utility of using multiple genetic markers (FitzSimmons et al. 1997; Diaz-Almela et al. 2004; VanOppen et al. 2001) because they provide a clearer indication of genome wide variation. For this reason we have chosen to build on our initial mitochondrial study with more detailed genetic surveys using microsatellite markers. Microsatellites are frequently referred to as simple sequence repeat (SSR) or variable numbers of tandem repeat (VNTR) loci composed of a sequence of bases repeated in tandem (Garza & Williamson 2001; Nakamura et al. 1987). They are considered simple because the repeats are comprised of 2-4 bases and therefore exist as either: "dimeric" (2), "trimeric" (3) or "tetrameric" repeat types, the prefixes correspond to the number of bases of each repeated unit. The number of times the unit is repeated varies from individual to individual. This variability is thought to occur because of strand slippage during DNA replication. Due to the great variability in the number of repeats in most loci, microsatellites have proven to be an important marker type for many areas of genetic research (Orti et al. 1997; Laloi et al. 2004; Luikart et al 1998; Pinho et al. 2004). Because of their high levels of variability (Jarne & Lagoda 1996), microsatellites have proved very useful in detecting: historical population size changes (Luikart et al 1998; Spencer et al. 2000; Beebee & Rowe 2001), population levels of genetic variability, population structure (Coifi & Bruford 1999; Fredsted et al. 2005), gene flow and potentially important conservation areas (Bouzat et al. 1998; Pinho et al. 2004; Orti et al. 1997; Whitehouse & Harley 2001). Consequently, the large number of alleles and high levels of heterozygozity typical of microsatellites will enable resolution of differences in genetic diversity among islands with contrasting isolation times and areas.

Several microsatellite loci have been already been isolated from closely related lizard species (Boudjemadi et al. 1999; Laloi et al 2004; Nembrini & Oppliger 2003; Pinho et al 2004; Richard & Thorpe 2000) as well as directly from the *P. erhardii* species (Poulakakis 2005; this study). In the present study, five microsatellite loci were obtained from either cross species amplification (two markers) or using primers designed specifically from loci isolated from *P. erhardii* (three markers).

Inbreeding, ectoparasite loads and Fitness

It has been proposed that genetic diversity at specific loci, as well as throughout the genome, is important in determining resistance to parasitism. Therefore, high levels of inbreeding, are expected to

compromise resistance to parasites. While parasite prevalence has been previously linked to impoverished levels of genetic diversity in some instances (Coltman et al. 1999; Luong et al. 2006), it is not so in other studies (Hale & Briskie 2007; Stevens et al. 1997). These discordant findings could be attributed to differences in breeding time, body condition, stress experienced by the host or other unknown ecological factors, all of which could affect ectoparasite load (Hawley 2007; Thomkins 2007). It is therefore of utmost importance that future studies attempt to correct for these variables.

Haematophagous parasites such as ticks and mites are associated with many animals and can damage host tissues, deplete fluids, trigger the immune response and serve as vectors of disease (Wakelin, 1996; Sorci et al. 1997) exerting a fitness cost on their host. Several studies have examined lizard species and their ectoparasites to determine what factors may be correlated with ectoparasite load. A study conducted on *Lacerta vivipara* found that whilst parasite load was negatively correlated with host density prevalence was unaffected suggesting that un-parasitized lizards did not avoid parasitized lizards (Sorci et al. 1997). A study conducted by Salvador et al. (1996) found that when testosterone levels of the common Spanish lizard, *Psammodromus algirus* was experimentally manipulated, males with higher testosterone had a greater susceptibility to ticks than non-manipulated males. A similar study conducted on Australian sand lizards also found that lizards that lost weight had greater tick loads compared to control males. These studies underline the importance of examining ectoparasite prevalence during breeding season, when testosterone levels are elevated, since the immune system of males is repressed.

For centuries local herdsmen have taken their livestock, primarily goats, to uninhabited islands to allow them to graze on the wild vegetation. It has been suggested that grazing is one of the harshest anthropogenic activity on the small islands of the Cyclades (Panitisa et al. 2006). Grazing can alter the vegetative landscape in several ways. First, it can alter the density of plants found on the islands, essentially reducing island plant biomass. Not all species, however, are likely to be affected in the same way. For example in the Aegean, halophyte species richness does not appear to be affected by grazing pressures (Panitisa et al. 2006). Although the authors offer no explanation for this finding, they do mention that halophytes, due to their ability to live in saline conditions, often thrive where other plants cannot live and presumably where sheep do not graze. Second, livestock introductions can bring seeds from plants found on the mainland adding new species to the islands. Third, livestock could potentially influence the distribution and abundance of parasite communities on these islands (Arthur 1973). Consequently, grazing and the introduction of livestock may impact not only plant species composition and richness (Panitisa et al. 2006) but also the prevalence of diseases and pathogens on island communities (Hoogstraal 1981; Altizer et al. 2001).

Major hypotheses

This study aims to address how island characteristics such as island age, island area and the distance from source populations affect the genetic diversity of *Podarcis erhardii*. Specifically, I predict that genetic diversity is positively correlated with island area and negatively correlated with island age. Since I suspect that gene flow does not influence the genetic diversity of our study species, I predict that the distance to the nearest source population should have no effect on the genetic diversity of *P. erhardii*. I expect that patterns of mitochondrial genetic diversity and phylogenetic history will reflect the fragmentation history of the island system.

Island area is often indicative of the amount of available resources (cover, food etc.) that island possesses. Consequently, larger islands support larger populations and may maintain greater genetic diversity than small populations. The smaller the population and the longer it remains small, the more genetic variation it will lose (Leberg 1992). Small populations face problems such as inbreeding and loss of rare and potentially novel alleles (Frankham 1996), both of which can increase the likelihood of extinction (Frankel and Soule 1981). By comparing an island's area to the genetic variation of its population, it is possible to quantify the loss of genetic diversity as a function of area. Microsatellite genetic diversity will be quantified by calculating (a) the average number of alleles per island per locus and (b) levels of unbiased heterozyogozity. Estimates of population divergence will be calculated using pairwise F_{st} estimates in order to test for isolation by distance effects. Previous studies have shown the utility of using average number of alleles (A) and mean ratio of the number of alleles to the range in allele size (M) with microsatellite data rather than heterozyogozity excess estimates (HE) alone (Garza & Williamson 2000). We will therefore calculate both A and M. I predict that average allelic number will be a more sensitive indicator of island area and age than average expected heterozygozity. We expect that M will be smaller in severely bottlenecked populations than populations that have not been subject to dramatic reductions in area.

Island age is a measure of time since isolation. Older islands have been isolated for a longer period of time and have been exposed to the cumulative effects of drift longer. We predict that older islands should be less genetically diverse than younger islands. In the absence of immigration, extinction is a driving force on continental land fragments. The population formed at island inception is assumed to have the same genetic composition as the adjacent landmass and any differences in allelic composition

and heterozygozity are expected to be the result of drift. However, mutation could potentially regenerate genetic diversity but only over relatively long periods of time (Frankham et al., 2002).

The distance from a source population should not have an effect on genetic diversity since *P. erhardii* has very limited dispersal capabilities. We therefore predict that genetic divergence will be random with respect to distance and we do not consequently expect to see patterns of isolation by distance. A previous study of land bridge islands has shown that elevation of the island (measured up from the sea floor, or depth between islands if measuring from sea level) was the most predictive indicator, while distance from island to mainland was the worst single indicator (Case 1975). As fragmentation history is believed to be the most important factor shaping historical differentiation among islands, I will also test for an effect of isolation by history using the maximum island depth separating the islands as a measure of distance instead of kilometers between islands. I predict that islands separated by shallower depths will have had a more recently shared history than islands separated by greater depths.

Finally, we predict that parasite burden will be positively correlated with both island age and level of inbreeding (*f*) and negatively correlated with allelic richness (A) and heterozygozity (He). This is because the older the island, the greater the duration of the population bottleneck experienced and the greater the loss in genetic variation.

To test these predictions we will quantify parasite burden on all islands and regress these values against both island age and island area. We will also regress parasite burden against *f*, the inbreeding coefficient to determine if there is a significant relationship between inbreeding and the parasite prevalence. Additionally we will examine the grazing histories of these islands to determine if there is a relationship between the severity of grazing practices and parasite burden, since grazing could provide a source of parasites and could therefore be an important predictor of parasite burden.

MATERIALS AND METHODS

Field sites

The Cyclade islands are located in the southeast Aegean Sea in Greece (Figure 5) and are arranged in a circular or cyclic formation, thus their name. Islets included in this study were geographically grouped into several clusters based upon a shared geologic history. The first cluster consists of the main island of Naxos, Paros and three neighboring islets (Ovriokastro, Makronissi, and Kopries), all within 20 kilometers of Naxos. The islands of Ovriokastro and Makronissi are much closer to Paros. However, samples could not be obtained from this island, as *P. erhardii* is extinct on Paros. The second cluster is centered on Keros southeast of Naxos and includes six adjacent islands (Koufonissi, Glaronissi, Daskalio, Megali Plaka, Andres and Antikeros). The third cluster is around the island of Iraklia to the south of Naxos and west of Keros and includes three islets (Schoinoussa, Agrilou and Megali Ambulas).

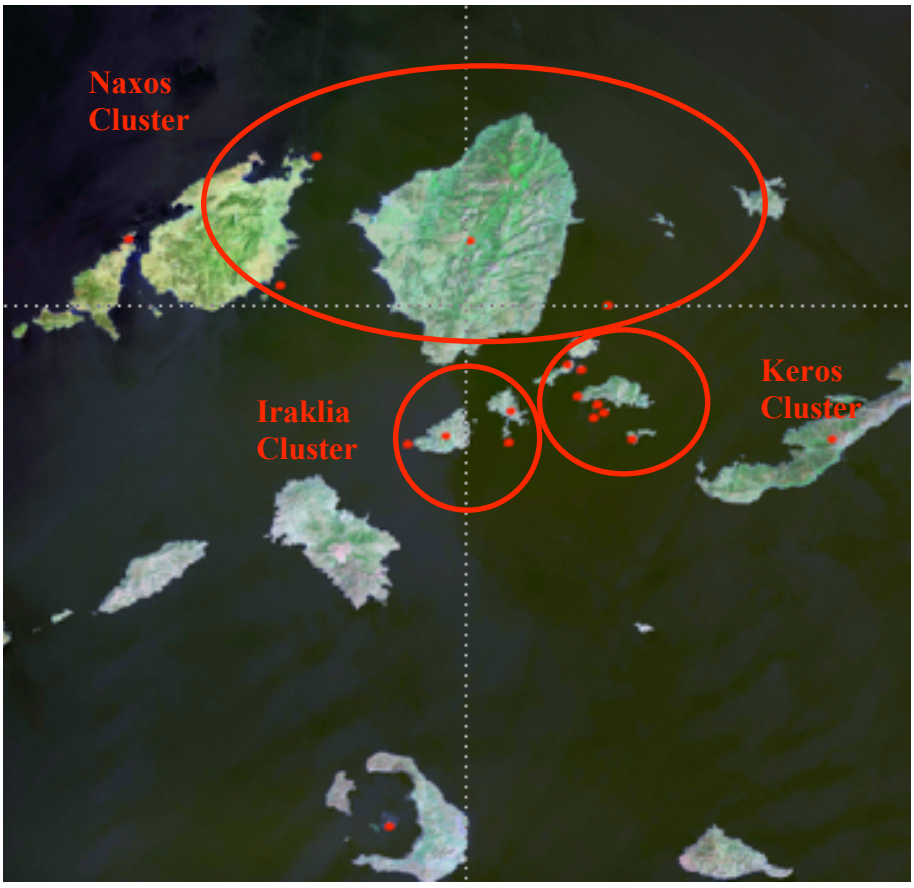

Figure 5: Islands sampled are given with a red dot, island clusters circled.

Table 1. Location, sample sizes and season lizards were sampled for the present study.

Island Name	Code	Sample Size	Field Season	Latitude	Longitude
Agrilou	Ag	30	2005	36.83292	25.527678
Amorgos	Am	20	2006	36.83972	25.897220
Andreas	An	25	2005	36.87111	25.638060
Antikeros	Ak	19 + 1*	2005 & 2006*	36.836973	25.669041
Chtapodia	Ct	7	2006	37.407778	25.564444
Choironissi	Xo	14	2006	37.369167	25.261944
Daskalio	Da	29 + 48*	2005 & 2006*	36.886211	25.605869
Donoussa	Do	13	2006	37.10472	25.812500
Phtira	Fi	21	2005	37.054766	25.085306
Glaronissi	Gl	27 + 2	2005 & 2006*	36.915382	25.607929
Iraklia	Ir	17	2006	36.83889	25.454440
Kato Revmatiaris	Kr	4	2006	37.393056	25.258889
Keros	Ke	15	2006	36.89111	25.647500
Kopries	Kp	27 + 55*	2005 & 2006*	36.986923	25.647240
Koufonissi	Ko	17	2006	36.92111	25.59278
Loumboudiaris	Lo	3	2006	36.867777	25.634722
Makronissi	Mk	23 + 6*	2005 & 2006*	37.005980	25.261002
Megalos Ambelas	Ma	25	2005	36.828180	25.411377
Megali Plaka	Mp	26	2005	36.876943	25.627756
Naxos	Nx	32	2005	37.059287	25.477638
Nea Kameni	Nk	32	2004	36.398626	25.398159
Ovriokastro	Ov	28	2004	37.151297	25.298853
Schoinoussa	Sk	21	2005	36.867536	25.528107

* Denotes second field season

Animal sampling

The bulk of the field work in 2005 was conducted from May 5th – May 31st, during the hours of 8:00 am and 6:00 pm. Additional samples were provided by collaborators Dr. Johannes Foufopoulos (University of Michigan, US) and Dr. Paniogitis Pafilis (University of Athens, Greece) from islands Nea Kameni and Santorini in 2004, and from islands Amorgos, Chtapodia, Choironissi, Donoussa, Iraklia, Kato Revmatiaris, Koufonissi and Loumboudiaris in 2006. Of these islands, only data from Nea Kameni, Santorini, Iraklia and Koufonissi are presented here.

Animals were captured using either silk nooses, sticky traps or by hand and were then placed into a cloth container where body measurements could be recorded in a shaded place. All animals were weighed in grams and measured (snout-vent length and head-tail length) in millimeters. Small tissue samples were obtained either through tail or toe clips or from autotomized tails and were stored in screw-top vial containing 100% ethanol (Sigma). A blood smear was taken from the tail to assess the identity and prevalence of blood parasites in future work. Whenever possible, blood-dots were obtained using a

clean piece of blot paper (Fisher) to provide an additional source of DNA in the event that the small tissue sample taken was not adequate for subsequent genetic analyses.

Ectoparasites were sampled with a pair of fine forceps and the number of ticks was counted for each animal. In the case of ticks, all were removed using forceps and placed in 100% ethanol in screw-cap vials for storage and subsequent identification. In the case of mites, it was impossible to judge the exact number of individuals so qualitative estimates of their prevalence (none, light, moderate, and heavy) were determined based upon parasite prevalence on each island. Based upon the number of parasites observed on each animal, a score of 0 was given if no parasites were present, 1 for light, 2 for moderate and 3 for heavy infestation. Prevalence was calculated by dividing the number of individuals infected with ectoparasites by the number of individuals sampled from that island. This value was then multiplied by 100 to yield a percentage value. An estimate of total parasite burden was computed by summing the mite and tick prevalence for each island. The parasite prevalence values as well as the total parasite burden values were then used for all linear regression analyses. Table 2 contains all parasite scores used in the linear regression analysis.

Table 2: Provides the parasite burden category as well the parasite prevalence for all islands where ecto-parasite burden was quantified.

Island	Mite Score	Mite Prevalence	Tick Score	Tick Prevalence	Total Parasite Burden
Agrilou	0	0.0	1	3.3	1
Andreas	3	4.0	3	100.0	6
Antikeros	2	19.0	2	63.1	4
Daskalio	1	6.6	0	0.0	1
Phtira	1	93.0	1	28.5	2
Glaronissi	0	0.0	3	96.2	3
Kopries	3	96.2	3	100.0	6
Makronissi	1	4.0	0	0.0	1
Megalos Ambelas	3	84.0	0	0.0	3
Megali Plaka	2	26.9	0	0.0	2
Ovriokastro	0	0.0	1	27.5	1
Schoinoussa	2	57.1	2	71.4	4

Animals were marked with a number using a non-toxic water based paint pen and released back into their territory following processing in order to avoid sampling the same animal more than once. This work was conducted in accordance with protocols laid out in the UNO (Protocol # 068) and University of Michigan (Protocol #09085) IACUC protocols.

Laboratory procedures

DNA extraction

DNA was extracted from the tissues using high salt, phenol-chloroform procedure outlined by Sambrook et al. (1989). Approximately 100mg of tissue from each animal was placed in 500µL of SNET buffer (Sambrook et al. 1989) composed of 1% SDS, 400 mM NaCl, 5 mM EDTA and 20 mM Tris·HCl, pH 8.0, with proteinase K (0.2 mg/ml). The tissue was homogenized with a hand-held eppendorf homogenizer and incubated at 55° C overnight. The following day, the DNA was extracted using a mixture of phenol: chloroform: isoamyl alcohol (25:24:1) and centrifuged at 14,000 rpm for 5 mins. Following centrifugation, the upper aqueous layer was removed and DNA was precipitated with 500µl of isopropanol. The sample was then washed once in 0.5mL 70% ethanol, centrifuged at 14,000 rpm and washed again with 0.5mL 100% ethanol (Fisher) before pelleting the DNA at 14,000 rpm for 10 mins. The ethanol was removed and the DNA pellet was then dried either on the bench top or in a SpeedVac for 5-10 mins. The DNA pellet was then resuspended in 20 µL of TE (Tris 10mM, EDTA 1mM pH 7.5), and RNAse (20µg/mL). The extractions were then stored at 4°C overnight prior to further analysis. All DNA extractions were run on a 1% agarose (Sigma) gel to verify the yield. DNA concentration of all samples was also quantified using a NanoDrop-1000 spectrophotometer (NanoDrop Technologies) and the number of nanograms per microliter (ng/µL) for each sample was recorded.

Mitochondrial cytochrome b analysis

A. PCR amplification

A 456 bp fragment of the mitochondrial cytochrome b locus was amplified by polymerase chain reaction (PCR) using universal primers (Palumbi 1996) that were modified to better match the lacertid cytochrome b data available in GenBank. The PCR was conducted in a BioRAD I-CYCLER using the following conditions: 1st step: denaturation for 3 minutes at 94ºC; 2nd step: 30 repeated cycles of denaturation for 30 seconds at 94°C, annealment for 30 seconds at 47°C and extension for 30 seconds at 72°C; 3rd step: a final extension for 10 minutes at 72°C; 4th and final step: 4°C hold for infinity. All reactions were conducted using Invitrogen reagents. The final concentration of the reaction reagents consisted of 1X enzyme buffer (200 mM Tris pH 8.4, 500 mM KCl), 1.5 mM Mg^{2+}, 2µM of each primer, 0.2mM dNTPs, 0.5U of *Taq* polymerase and 15-30 ng DNA per 50uL reaction. Following PCR amplification, 10µl of the reaction was run on a 1.6% agarose gel to check the yield and verify the size of the product using a 1 kb plus ladder (Invitrogen). The gels were then stained in an ethidium bromide (0.1µg/mL) solution suspended in 0.5X TBE and the PCR products were visualized using the BioRad GelDoc system and ChemiDoc software. PCR products were then cleaned using GeneClean

Turbopurification kit (QBiogene), following manufacturer's guidelines. Cycle sequencing reactions were then conducted using 0.8pmol of each primer, 1X Buffer and 1μL BigDye (Applied Biosystems) in a total volume of 5μl. The sequencing reaction was then cleaned by passing it through a Sephadex column (Type G50, Fisher) and read on an automated sequencer (ABI).

B. Cytochrome b analysis

The chromatograms generated from the ABI Prism 3100 were edited using Sequencher version 3.01 (Gene Codes Corp., Ann Arbor, Michigan USA) and aligned in Clustal X (Thompson et al 1997). The program DNAsp (Rozas et al. 2003) was used to calculate the average number of nucleotide differences per site, or nucleotide diversity, π for each island population (Nei 1987, equation 10.7). ARLEQUIN version 3.0 (Excoffier et al., 2005) was used to obtain estimates of pair-wise genetic distance Φst between islands. The program TCS version 1.21 (Clement et al. 2000) was then used to calculate a network tree between all mitochondrial haplotypes with a 95% maximum parsimony criteria. ModelTest (Posada & Crandall 1998) was used to determine the model of nucleotide substitution that best fits the data.

Analyses for phylogenetic inference of mitochondrial cytochrome b data were conducted using three methods: neighbor joining (NJ), maximum parsimony (MP) and maximum likelihood (ML). MEGA (Kumar et al. 2004) was used to construct a neighbor joining tree with 1000 bootstrap replicates. The MP analysis was performed using PAUP (Swofford 1999) using the heuristic searches option, the stepwise addition option and tree-bisection-reconnection (TBR) branch swapping algorithm. The strength of support in nodes was assessed by 1000 bootstrap replicates. PAUP was also used for the ML analysis. Model parameters were based on the best-fit model of substitution determined using MODELTEST (version 3.06, Posada and Crandall 1998). Heuristic searches were performed using stepwise addition and TBR branch swapping algorithm. Due to the complexity of a ML search only 100 replicates were employed for the bootstrap consensus tree.

Microsatellite loci

A. *Construction of a microsatellite library from P. erhardii*

Microsatellite isolation was carried out using a protocol outlined by Hammond et al. (1998). Genomic DNA (~6μg) from several animals from Nea Kameni and Santorini was digested with the enzyme *Mbo*I (New England Bio-labs) overnight at 37ºC. Digested fragments of the desired size (300-800 bp) were excised from a 2% agarose gel using Turbo GeneClean Spin Kit (QBioGene) following the manufacturer's protocol. After extracting the DNA from the gel, *Sau*lA and *Sau*lB linkers were ligated to the size selected fragments. The fragments were then amplified via PCR and those fragments containing the desired repeats were enriched by dimeric-repeat specific biotinylated probes specific to the desired microsatellite repeat of interest. Subsequently, a plasmid library was constructed by ligating the microsatellite enriched fragments into (*E. coli*) competent cells using the TA cloning vector 3.1 (Invitrogen). Colonies were grown on LB agar plates containing ampicillin (4mg/mL), thus selecting for colonies that were transfected with the plasmid. Clones were then screened for the presence or absence of a PCR insert via blue-white color selection using the compound X-Gal (D-galactopyranoside). Whereas white colonies contain the PCR insert, blue colonies do not. This is because the insertion of the PCR amplified DNA product into the plasmid disrupts the reading frame for beta-galactosidase (LacZ gene), thus preventing transfected cells from breaking down the X-gal substrate and turning blue. Therefore, by picking white cell colonies, only plasmid transfected cells containing an insert should be selected.

Plasmid DNA was extracted by using either a standard alkaline cell lysis protocol (Bimboim & Doly 1979) or in the case of 96 well formats, using the PureLink 96 Plasmid Purification System (Invitrogen) following the manufacturer's protocol. Plasmid inserts were then amplified using M13 primers directed against the flanking sequences and were screened on the basis of insert size. Inserts that were larger than 500 base pairs were subsequently selected for turbopurification (GeneClean) and were subsequently sequenced with M13 primers using ABI Big Dye Cycle Sequencing kit. Primers were then designed from sequence immediately flanking microsatellite inserts after the removal of the SaulA/SaulB linkers and the vector sequence. Of the eight microsatellite loci identified from our library screens, only three could be amplified predictably, of which only one was sufficiently polymorphic for subsequent analyses (T434).

B. *Cross-amplification of microsatellites from other species*

Primer pairs were obtained from current literature (Table 3) for species closely related to *P. erhardii.* Of the 27 loci examined, only primer pairs flanking 12 candidate loci (Lv319, Lv2145, Pb10, PmA7, PmB7, PmC8, PmD1, PmC24, Pb10, Pb20, Pb50, Pb66) amplified fragments of the expected size. These 12 candidate loci were then screened for polymorphism using PolyAcrylamide Gel Electrophoresis (PAGE). Of these, only six loci were found to be variable. These six loci were then sequenced to verify the presence of a microsatellite and only 5 were found to contain microsatellite repeats. To survey for microsatellite variation in lizard populations at these five loci, one primer from each pair was fluorescently tagged with either 6-FAM, HEX (IDT DNA Technologies) or NED (ABI) at the 5' end. Fluorescently labeled PCR products were then screened using the ABI 3100 prism and visualized using either GeneScan version 3.7 and Genotyper version 3.6, or Genemapper version 4.0 (ABI) software.

C. *Multiplex reaction*

Primer pairs from all five loci were combined in a single multiplex reaction using a multiplex kit (Qiagen) according to the manufacturer's guidelines. Following amplification via PCR, 0.50-1.0μL of the PCR product was combined with 11μL of formamide (Fisher), denatured (2 mins at 95°C) and then held on ice before loading on to the ABI Prism 3100 sequencer.

Table 3: Microsatellite loci assessed for amplification success in *P. erhardii*. Those in bold are those that were polymorphic and subsequently optimized for use in the present study.

Locus	Spp. Origin	Repeat	Exp. size (bp)	Source
Lv2145	*Lacerta vivipara*	$(TG)_{20}$	297-325	Boudjemadi et al. 1999
Lv472	*Lacerta vivipara*	$(AC)_{18}$	103-142	Boudjemadi et al. 1999
Lv319	*Lacerta vivipara*	$(AC)_{22}$	103-142	Boudjemadi et al. 1999
Lv4α	*Lacerta vivipara*	$(AC)_{14}A(GA)_{11}(CAGAG)_9(AG)_3$	114-139	Boudjemadi et al. 1999
Lv4x	*Lacerta vivipara*	$(GT)_{22}$	130-223	Boudjemadi et al. 1999
Lv4115	*Lacerta vivipara*	$(CA)_{18}$	114-156	Boudjemadi et al. 1999
A348	*Gallotia galloti*	$(AC)_{19}$	228	Richard & Thorpe 2000
A49	*Gallotia galloti*	$(CA)_{10}$	198	Richard & Thorpe 2000
B81	*Gallotia galloti*	$(TC)_{19}$	163	Richard & Thorpe 2000
B967	*Gallotia galloti*	$(GT)_3AT(GT)_{10}$	149	Richard & Thorpe 2000
B821	*Gallotia galloti*	$(AC)_{12}$	261	Richard & Thorpe 2000
PmC9	*Podarcis muralis*	$(CAA)_7CCA(CAA)_3$	130	Nembrini & Oppliger 2003
PmB7	*Podarcis muralis*	$(AG)_{19}$	129	Nembrini & Oppliger 2003
PmA7	*Podarcis muralis*	$(GT)_{18}$	182	Nembrini & Oppliger 2003
PmB4	*Podarcis muralis*	$(AG)_{16}$	135	Nembrini & Oppliger 2003
PmC24	*Podarcis muralis*	$(TAAA)_3(CAA)_{10}(ATA)_3$	205	Nembrini & Oppliger 2003
PmC8	*Podarcis muralis*	$(CAA)_8$	135	Nembrini & Oppliger 2003
PmD1	*Podarcis muralis*	$(CTT)_{16}$	134	Nembrini & Oppliger 2003
P7	*Podarcis erhardii*	$(CA)_6$	83	T. Crane Msat library
P8	*Podarcis erhardii*	$(GT)_{12}$	92	T. Crane Msat library
P13	*Podarcis erhardii*	$(AC)_{14}$	156	T. Crane Msat library
P15	*Podarcis erhardii*	$(AC)_{12}$	237	T. Crane Msat library
P16	*Podarcis erhardii*	$(CA)_7$	126	T. Crane Msat library
T429	*Podarcis erhardii*	$(TG)_{11}$	169	T. Crane Msat library
T430	*Podarcis erhardii*	$(TG)_{14}$	137	T. Crane Msat library
T434	*Podarcis erhardii*	$(TG)_{11}$	131	T. Crane Msat library
Pb10	*Podarcis bocagei*	$(GT)_N GC(GT)_N$	178-204	Pinho et al. 2004
Pb11	*Podarcis bocagei*	$(TG)_N$	152-180	Pinho et al. 2004
Pb20	*Podarcis bocagei*	$(AC)_N$	124-155	Pinho et al. 2004
Pb37	*Podarcis bocagei*	$(CA)_N$	129-158	Pinho et al. 2004
Pb47	*Podarcis bocagei*	$(GT)_N$	203-238	Pinho et al. 2004
Pb50	*Podarcis bocagei*	$(CA)_N$	113-135	Pinho et al. 2004
Pb55	*Podarcis bocagei*	$(TG)_N$	228-242	Pinho et al. 2004
Pb66	*Podarcis bocagei*	$(TG)_N$	138-171	Pinho et al. 2004
Pb73	*Podarcis bocagei*	$(CA)_NCT(CA)_N$	146-178	Pinho et al. 2004
Pod1a	*Podarcis erhardii*	$(TC)CTTG(TC)_3$	128-144	Poulakakis et al. 2005
Pod1b	*Podarcis erhardii*	$(AC)_{12}(CA)_7$	138-182	Poulakakis et al. 2005
Pod2	*Podarcis erhardii*	$(TC)_{16}TA(TG)_{10}$	90-128	Poulakakis et al. 2005
Pod3	*Podarcis erhardii*	$(ACCCC)_3N_{14}(TG)_4N(GA)_{12}(TG)_{14}$	122-178	Poulakakis et al. 2005
Pod8	*Podarcis erhardii*	$(CT)_{18}$	177-213	Poulakakis et al. 2005

Analysis of microsatellites

Multi-locus genotypes were analyzed using Genemapper version 4.0 (ABI). In order to classify alleles, bin sets were created by repeatedly re-running individuals with known multi-locus genotypes. These bin sets correspond to the expected range in size, based upon the range in allele size, optimized to minimize the variance about the mean (Dury and Cardon 1997). In initial runs, the average amount of variance observed from repeatedly running the same allele was between 0.007 – 0.010 bp^2 (or a standard deviation of 0.084 – 0.1 bp). The variance from one run to another was found to be negligible relative to the base pair differences between alleles (2bp), thus it is possible to be confident that alleles were called correctly. Table 4 provides the amount of variance observed for each allele when the sample Nk29 was repeatedly run.

Table 4: The variance observed from running the same sample (Nk29) 10 times, for each of the microsatellite loci examined in the present study.

Locus	Variance (σ^2)	Number of Replicates
Lv319	.007	10
Pod1a	.010	10
Pod8	.008	10
T434	.010	10

In order to minimize the amount of genotyping error in the data, the following precautions were taken (Bonin et al. 2004): (1) a subset of individuals were run through the ABI Prism 3100 numerous times (at least twice) to check for repeatability of scoring and search for any allelic dropouts; and (2) two different individuals were used to score the same alleles.

Following the initial examination of variance, genotype profiles were exported as .txt files and imported into Excel where the macro implemented in the program Flexibin (Amos et al. 2006) was used to determine bin limits and calculate the observed mean values + S.D. for every allele. To establish allele bins, the user inputs raw base pair calls obtained from the Genemapper software and the program then parses through the raw data determining the number of repeats contained within each allele, the frequency of each allele and standard deviation observed for each allele bin. When determining bins, all replicate genotypes were used, so if an individual was run numerous times that allele (with slightly different values) was included. This binning was carried out in order to obtain better estimates of the amount of variation observed for every allele and to better assess the potential for mis-classification of alleles. If alleles are binned correctly the standard deviation observed will be less than 0.35 (Amos 2006).

After alleles were binned and multi-locus genotypes were obtained for all individuals, the data were copied and pasted into another worksheet, where a macro implemented in Microsatellite Toolkit version 3.1 (Park 2001) was used to parse the data, provide an overview of allele frequencies and observed heterozygozities for each island and export multi-locus genotypes in various formats for subsequent analysis in other software packages.

ARLEQUIN version 3.1 (Excoffier 2005) was used to calculate pairwise Φst estimates between island populations, test for linkage disequilibrium between loci and detect deviations in Hardy-Weinberg equilibrium within islands. In conjunction with this, the program GenAlEx6 (Peakall & Smouse 2006) was also employed to estimate deviations from Hardy Weinberg equilibrium and obtain allele frequencies at each locus for individual islands. ARLEQUIN was also used to calculate haplotype diversity (H) using the formula: $H = \frac{n}{n-1}\left(1 - \sum_{i=1}^{k} p_i^2\right)$, where n is the sampled size and k is the number of haplotypes.

The web based version of GENEPOP (http://genepop.curtin.edu.au) (Raymond & Rousset 1995) was used to determine Weir and Cockerham's f (1984), a measure of inbreeding equivalent to Wright's F_{IS}. Weir and Cockerham's f is based on the proportion of allelic variance in a subpopulation that is contained within an individual and is given by the following formula: $1-f = c/(b+c)$, where b is the amount of variance between individuals and c is the amount of variance between gametes within an individual. Unlike other F statistics proposed by Nei (1976), Weir and Cockerham's method is not affected by sample size.

E. Isolation by distance analyses

A Mantel test was conducted using the isolation by distance (IBD) web service IBDWS (Jensen et al 2005). First a genetic distance matrix was calculated using Arlequin (Φ_{st} for mitochondrial DNA) and GenAlEx6 (F_{ST} for nuclear DNA). The geographic distance between islands was obtained from the Great Circle calculator (www.gb3pi.org.uk/great.html) using the Latitude and Longitude coordinates given in Table 2. The depth between islands was inferred from bathymetric data. The depth estimate was determined using isobaths (underwater land curves), the value obtained corresponded to the amount of water that would have to be removed for the two islands being compared to connected to one another. IBDWS then conducted a matrix correlation test and outputs a scatter plot of pair-wise genetic versus geographic distances. The significance of this association was assessed by bootstrapping pseudoreplicates

sampled from the data. The strength of the relationship was assessed by examining the R^2 value obtained through reduced major axis regression implemented in the program.

F. Statistical analysis

Multiple linear regression analysis (also known as a multi-predictor analyses) was used to determine what proportion of the variation in each of the following response variables: heterozygozity (He), average number of alleles (A), M, *f*, tick prevalence, mite prevalence and total parasite burden) was explained by the following predictor variables: island age, area and the interaction between age and area. Island area was log transformed to normalize the distribution of area values (Vittinghoff et al. 2005). Box plots were generated for each predictor variable to ensure that values did not deviate substantially from a normal distribution. Additionally for each linear regression analysis, the residuals of the analysis were plotted against the predictor variable to check for homogeneity of variance and verify that the data fit a linear model.

A multi-predictor regression model was constructed for each response variable, where the predictor variables age, area and the interaction term were included in the initial model and retained if they were significant ($p < 0.10$). For each model, the coefficient of determination, R^2 was obtained. R^2 is useful when comparing several models, because it represents the proportion of the total variance observed in the model that can be attributed to one or more predictor variables (Vittinghoff et al. 2005).

A linear regression model was constructed for each of the parasite response variables, using the grazing score (determined in table 1) as the predictor variable. Parasite variables were also regressed against the genetic measures of He, A, M and *f* in order to examine whether genetic measures could adequately predict levels of parasite burden.

To test the hypothesis that grazing may potentially impact the abundance of parasites on many of the islands included in this study, the severity of grazing pressure was classified according to three criteria: a) presence of animal dung, b) evidence of structures to protect animals, c) live animals or carcasses present. Each island received a score ranging from 0-3. If none of these criteria were met, the island received a score of 0. For every additional criterion, a score of 1 was added, with the highest score possible being 3. The grazing score was used for subsequent linear regression analyses using parasite explanatory variables (mites score, mite prevalence, ticks score, tick prevalence and total burden). Table 5

displays the criteria values and the grazing scores determined for each island where grazing pressure was assessed.

Table 5: Grazing criteria values and scores for each island determining the grazing severity

Island Name	Dung	Structures	Animals	Score	Grazing Severity
Agrilou	1	0	0	1	Light
Andreas	1	1	1	3	Heavy
Antikeros	1	1	1	3	Heavy
Daskalio	0	0	0	0	Light
Phtira	1	1	1	3	Heavy
Glaronissi	1	1	1	3	Heavy
Iraklia	1	0	0	1	Light
Keros	1	0	1	2	Heavy
Kopries	1	0	1	2	Heavy
Koufonissi	1	0	0	1	Light
Makronissi	0	0	0	0	Light
Mega Ambulas	1	0	0	1	Light
Megali Plaka	0	0	1	1	Light
Naxos	1	1	1	3	Heavy
Nea Kameni	0	0	0	0	Light
Ovriokastro	1	0	1	2	Heavy
Schoinoussa	1	1	1	3	Heavy

RESULTS:

Mitochondrial variation

A 430bp fragment of the mitochondrial cytochrome b gene was sequenced from 173 samples of *P. erhardii*. These samples were obtained from 17 islands distributed throughout the Cyclades. Only 16 unique haplotypes were obtained and many island populations were fixed for a single mitochondrial haplotype. Table 6 lists the sample size, number of haplotypes, haplotype diversity and nucleotide diversity (π) for each island. The following islands contained more than two haplotypes: Antikeros, Daskalio, Iraklia, Keros, Kopries, Koufonissi, Naxos and Nea Kameni. When cytochrome b sequences from a previous study of *P. erhardii* (Poulakakis et al., 2003) were combined with those obtained from the present study, the resulting database yielded a total of 58 unique haplotypes. Nine of the seventeen islands had no haplotype diversity (H = 0). The three islands with the greatest amount of haplotype diversity were Naxos, Nea Kameni and Iraklia, all relatively large islands. In contrast, almost all small islands, excluding the young island of Daskalio and the old island of Kopries, had no haplotype diversity. The measure of nucleotide diversity (π) within islands demonstrated that there was very little sequence divergence among haplotypes on most islands, an average value of only 0.003, with the greatest value being that of Nea Kameni (0.03552).

Table 6: Sample sizes, number of mitochondrial haplotypes, haplotype diversity, and π, a measure of nucleotide diversity for each population examined.

Island	Sample size	Haplotypes (#)	Haplotype diversity (H)	π
Agrilou	12	1	0.00000	0.00000
Andreas	10	1	0.00000	0.00000
Antikeros	13	2	0.15384	0.00036
Daskalio	12	2	0.16667	0.00078
Phtira	5	1	0.00000	0.00000
Glaronissi	10	1	0.00000	0.00000
Iraklia	5	2	0.60000	0.00280
Keros	5	2	0.20000	0.00047
Kopries	11	2	0.18182	0.00042
Koufonissi	5	2	0.40000	0.00280
Makronissi	9	1	0.00000	0.00000
Megali Plaka	15	1	0.00000	0.00000
Megalos Ambelas	14	1	0.00000	0.00000
Naxos	15	5	0.62857	0.00511
Nea Kameni	11	3	0.61718	0.03552
Ovriokastro	7	1	0.00000	0.00000
Schoinoussa	9	1	0.00000	0.00000

Phylogenetic analysis

The neighbor-joining bootstrap consensus tree was conducted using Mega 3.1 using 1000 replicates (Figure 5). For the reduced dataset used in maximum likelihood (ML) analysis, Modeltest identified HKY+G as the most appropriate model, with a transition to transversion ratio of 10.7425. The nucleotide frequencies observed (G = 0.12230, A= 0.27220, T = 0.31040, and C = .29510) were comparable to those reported previously for *P. erhardii* in Poulakakis (2003). ModelTest also identified an alpha shape parameter of 0.0127 with the proportion of invariant sites set to zero. ML searches were performed using TBR branch swapping with 100 bootstrap replicates. The maximum likelihood tree for cytochrome b is shown in the appendix (Figure A).

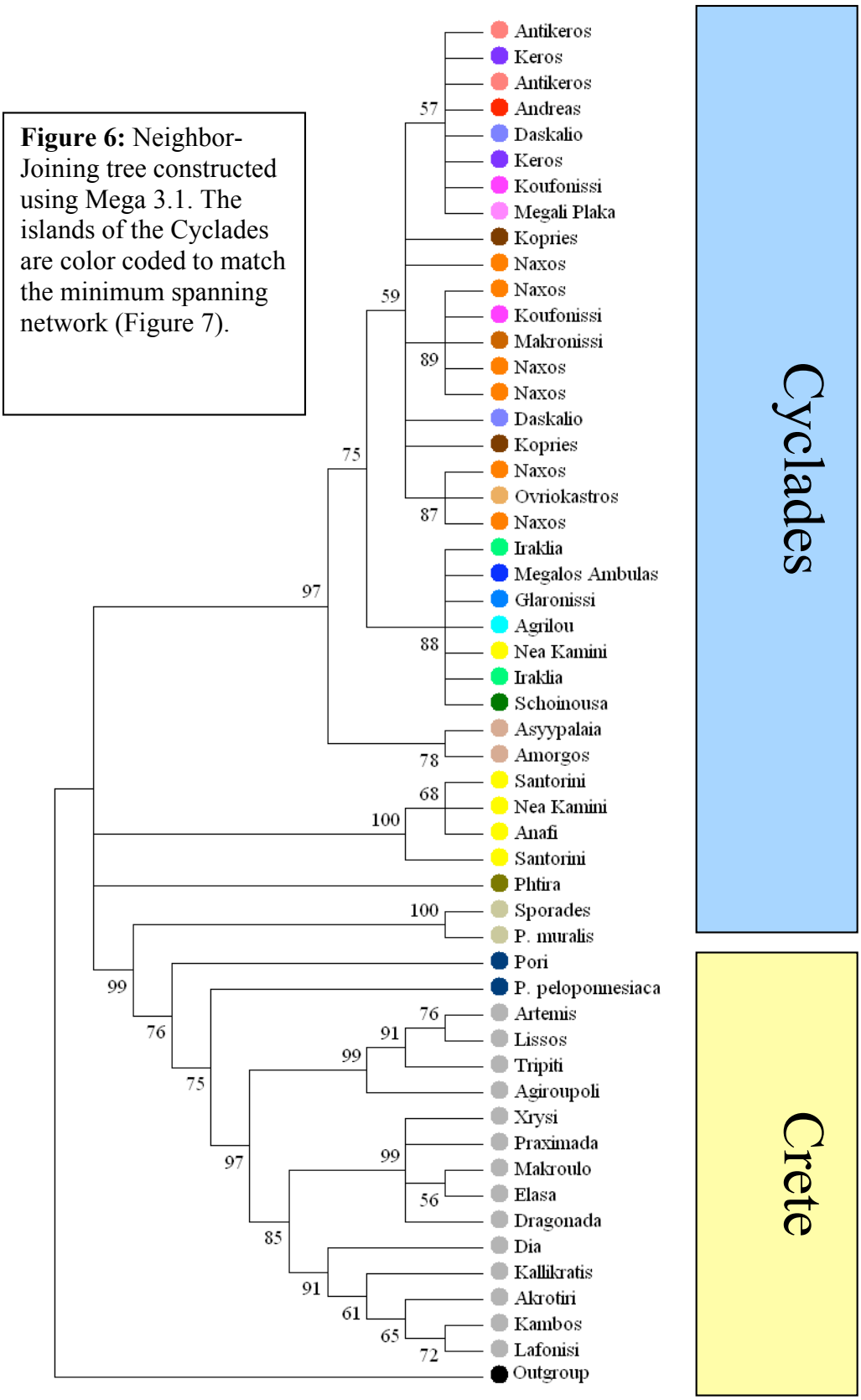

Figure 6: Neighbor-Joining tree constructed using Mega 3.1. The islands of the Cyclades are color coded to match the minimum spanning network (Figure 7).

Phylogenetic analysis of this combined dataset using any of the three different methods resolved two geographically distinct lineages: 1) *P. erhardii* populations from Crete (yellow), Pori and *P. peloponnesiaca* from the southern tip of the Greek mainland 2) populations of *P. erhardii* from the Cyclade islands (Blue). *P. erhardii* is paraphyletic with *P. pelonnesiaca* occupying a position between *P. erhardii* populations from Crete and those of Pori (See Poulakakis et al., 2003).

The Cyclades haplogroup shows a greater amount of sub-structuring than previous analyses revealed (Poulakakis et al. 2003). This is probably due to more extensive sampling efforts within islands and the addition of previously un-sampled islands. Within the focal clade, several regional clusters of island haplotypes were observed, with several haplogroups resolved with moderate bootstrap support.

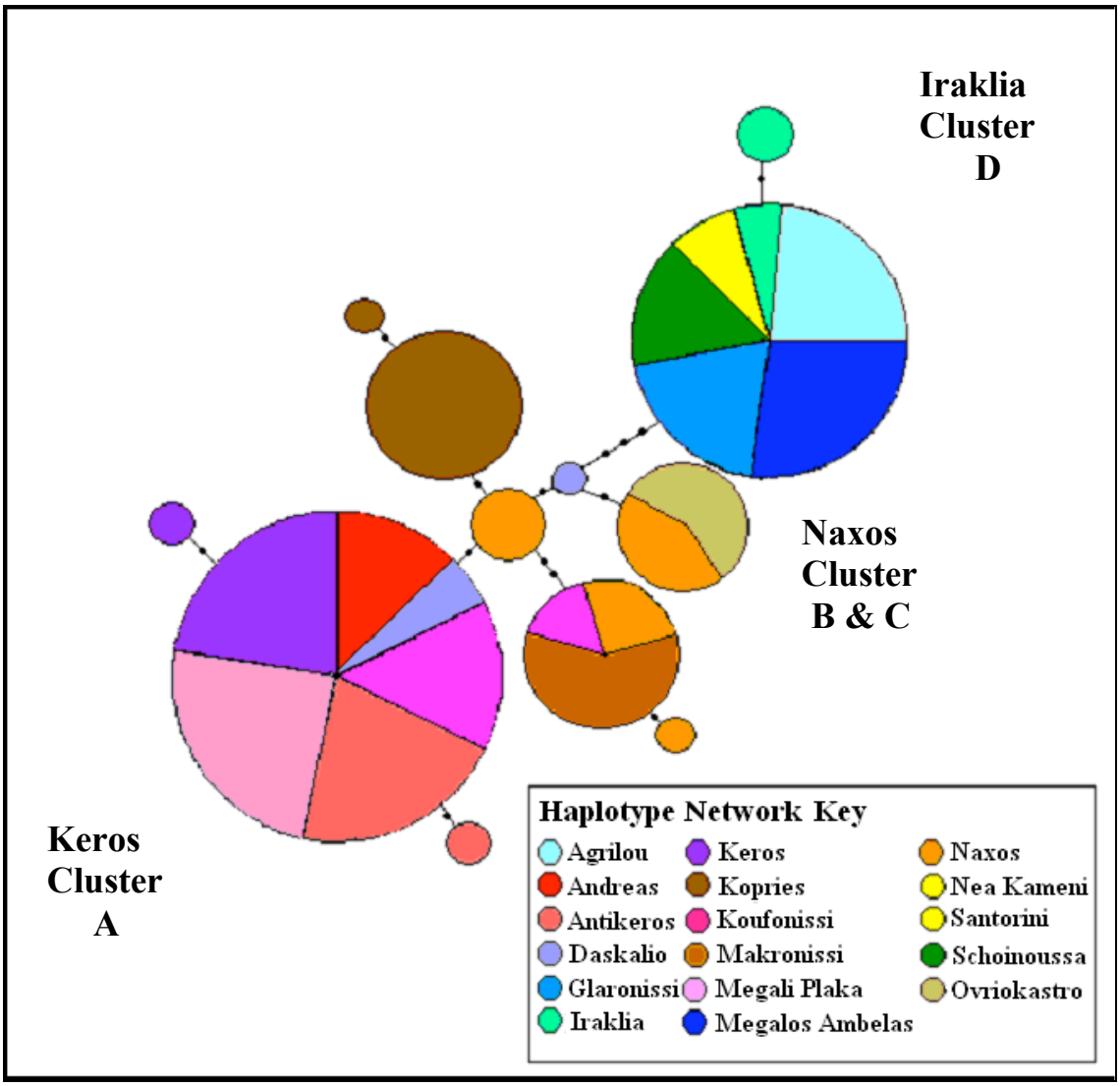

Figure 7: Minimum spanning network for a 430 base pair region of cytochrome b. Each circle represents a unique haplotype, the size of which is proportional to its frequency.

The 95% parsimonious haplotype network tree (Figure 7) constructed using TCS provides some evidence for regional structuring, albeit incomplete. First, haplotypes from the Eastern portion of the Cyclades (Keros, Antikeros, Koufonissi, Andreas, Megali Plaka and Daskalio) cluster into one haplogroup (A). Second, islands adjacent to Naxos (Makronissi, Kopries and Ovriokastro) tend to share similar haplotypes (B and C). However some islands have haplotypes distributed in different parts of the network, specifically Naxos, Kopries and Daskalio, which could be indicative of widespread fixation of ancestral polymorphism, gene flow or both. Third, there is another cluster (D) that includes the islands of Agrilou, Glaronissi, Iraklia, Megalos Ambelas, Schoinoussa and interestingly a few individuals from Kameni. These associations are consistent with the island clusters outlined in Figure 5, with the exception

of Glaronissi, which shares a common history with the Keros cluster but shares a haplotype with the Iraklia cluster.

There is also a group of haplotypes from Nea Kameni and nearby Santorini that are so divergent that they were not connected to the network because they were greater than the maximum number of changes allowed for reconstructing connections with 95% confidence. Haplotypes from Amorgos and Astypalia were grouped with one another but were not joined to the Cyclades. The two haplotypes from the island of Pori joined with one another only and were excluded from the Cyclades network. Also, the haplotype found on the island of Phtira were too divergent to be connected to the Cyclades network.

Microsatellite variation

The five microsatellite markers chosen for this study all contained di-meric nucleotide repeats. PCR amplified microsatellite loci were fluorescently tagged with three different colors to allow discrimination of co-amplified microsatellite loci. There was little overlap between adjacent alleles with the exception of Lv319 and Pod1a. In this case, care was taken to correctly assign peaks to individual loci and identify pull up artifacts between loci. The number of alleles observed as well as their size ranges, fluorescent label and repeat type are given in Table 7. The most variable microsatellite locus was Pb10 with 24 alleles, while the least variable was Pod1a with only 6.

Table 7: All microsatellite markers used in the present study, the total number of alleles observed, the size range in base pairs, the color and repeat type for each locus.

Locus	Total # of Alleles	Size Range	Color	Repeat type
Lv319	17	120-152	Green (HEX)	CA
Pb10	24	201-249	Green (HEX)	TC
T434	11	163-183	Yellow (NED)	CT
Pod1a	6	135-143	Blue (6-FAM)	GT
Pod8	22	176-212	Blue (6-FAM)	AC

Tests for correct binning

The graphical results represent a cumulative length display in base pairs for each locus (Figure B -D in the appendix). Each allele is shown in a different color for clarity. There were two cases of possible mononucleotide repeats, such as in the case of locus Pod8 where two suspect alleles at 201 and 205bp were identified (Figure 8). Individuals with these alleles were repeatedly analyzed with individuals of known repeat size one base pair above and below (i.e. 200, 202, 204, and 206 bp) to confirm the authenticity of these mononucleotide mutations. Once verified, these cases were then treated as novel alleles in all subsequent analysis.

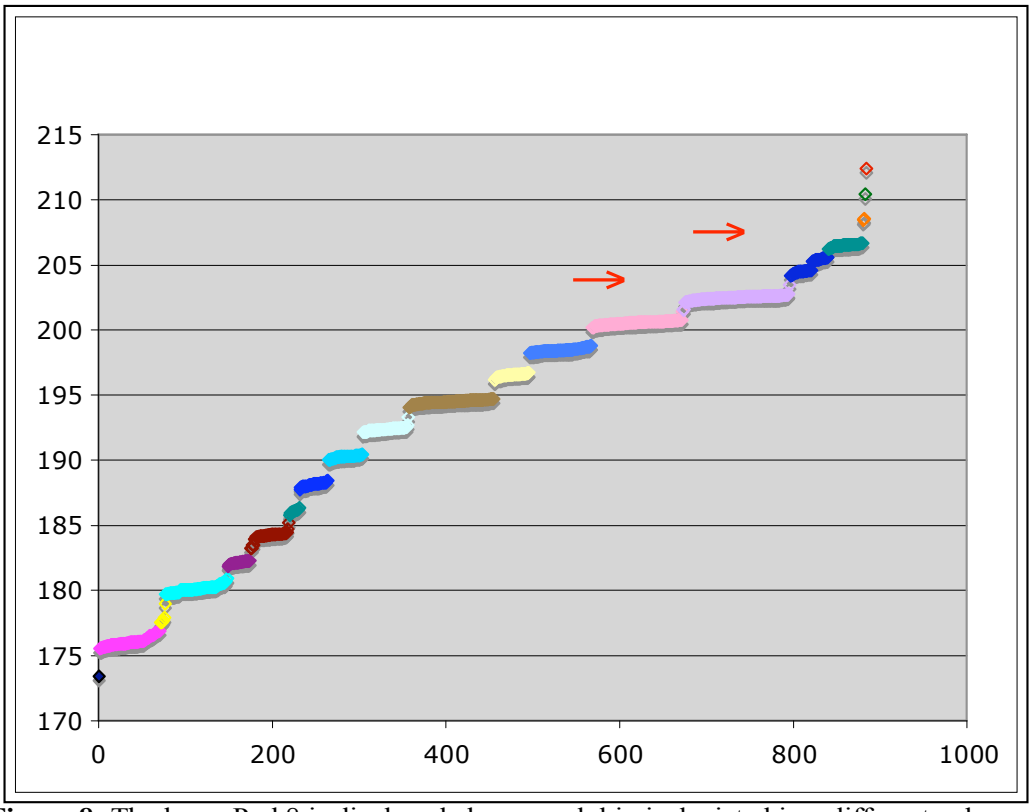

Figure 8: The locus Pod 8 is displayed above, each bin is depicted in a different color. The two questionable alleles (possible mono-nucleotide repeats) are pointed to with a red arrow. Similar graphs for all other loci can be found in the appendix (Figures B-E)

For each locus, the average size of each allele and its standard deviation are reported in Appendix Tables A-D. Table 8 illustrates an example of the summary statistics generated by the program Flexibin for locus Pod 8. Once mono-nucleotide repeats have been identified, standard deviations for each locus falls below an acceptable threshold of 0.35 SD. This binning process identifies any potential genotyping errors based on miscalling of alleles and resolves one base pair mutations.

Table 8: Allelic variance for Pod8, repeat numbers, expected size, observed size and standard deviation for each allele is given, as are the number of counts for each allele. *Mono-nucleotide repeats

Repeats	Length	Mean size (bp)	Std. Deviation	Count
1	176.02	176.06	0.340	70
2	178.06	177.89	0.135	5
3	180.10	180.10	0.271	72
4	182.14	182.11	0.095	26
5	184.18	184.17	0.325	44
6	186.22	186.06	0.146	12
7	188.26	188.10	0.152	33
8	190.30	190.23	0.113	40
9	192.34	192.36	0.166	53
10	194.38	194.46	0.136	98
11	196.42	196.51	0.126	41
12	198.46	198.46	0.139	72
13	200.50	200.54	0.158	102
13.5*	201.52	201.49	0.073	3
14	202.54	202.46	0.175	120
15	204.58	204.43	0.015	25
15.5*	205.60	205.53	0.017	104
16	206.62	206.48	0.103	40
17	208.66	208.48	0.092	2

Deviations in Hardy-Weinberg Equilibrium (HWE)

The Hardy-Weinberg exact test was employed to test for deviations between the expected and observed levels of heterozygozity. In addition to this the program, GenAlEx 6 was used to determine deviations from Hardy Weinberg equilibrium using a Chi-squared test. Following both sets of analyses a Bonferonni correction was employed to determine the appropriate table-wide appropriate critical value for rejection of the null hypothesis. For table-wide comparisons, this is equal to a p value of 0.0006.

There were 8 instances of significant ($p<0.0006$) deviation from HWE, all of which were due to heterozygote deficiency. Of these, 6 cases were observed for T434, 1 case for Lv319 and 1 case for Pb10. T434 makes a strong case for non-amplifying alleles, while the deviations observed for Lv319 and Pb10 could be due to selection or simply by chance. Table 9 provides an overview of the loci that significantly deviated from HWE as well as the island populations where deviations were observed.

Table 9: Loci that significantly deviated from HW Equilibrium ($p<0.0006$), the population that it was found in and the type of deviation observed. See the appendix (Figure E) for a complete list of observed and expected values of heterozygozity for each locus within each population

Locus	Population	Type of Deviation
Lv319	Ovriokastro	Heterozygote Deficiency
T434	Andreas	Heterozygote Deficiency
	Glaronissi	Heterozygote Deficiency
	Keros	Heterozygote Deficiency
	Makronissi	Heterozygote Deficiency
	Megali Plaka	Heterozygote Deficiency
	Naxos	Heterozygote Deficiency
Pod8	Naxos	Heterozygote Deficiency

However, for T434 the possibility of null alleles appears to be a more likely explanation for the observed deviations from HWE. Null alleles can result from a mutation in the DNA template sequence that interferes with the 3' binding site of one of the primers used to amplify the microsatellite locus. If this occurs then a heterozygote deficiency is observed because one or more alleles fail to amplify resulting in "false" homozygote genotype (Pemberton 1995).

Linkage disequilibrium (LD) or non-random association of alleles can arise as a consequence of mutation, random genetic drift, selection on single or linked alleles, and population admixture (Hartl and Clark 1990). Only two islands (Andreas and Agrilou) exhibited significant LD after Bonferroni correction (Figure 7, 8). On the island on Andreas, linkage disequilibrium was detected between Lv319 & T434, and Lv319 & Pb10. On the island of Agrilou linkage disequilibrium was detected between Lv319 and Pod8. Histograms representing the loci where linkage disequilibrium was detected after Bonferroni correction on the islands of Andreas and Agrilou are presented in the appendix (Figures F&G).

Of the 17 islands, we found that 12 contained 100% polymorphic loci**.** The percent of polymorphic loci ranged from 40% to 100%. The island populations with less than 100% polymorphic loci were: Phtira, Keros, Megali Plaka, and Ovriokastro. Phtira had the lowest number of polymorphic loci (3 out of the 5 loci scored were monomorphic). While Keros, Makronissi, Megalos Ambelas and Ovriokastro all were 80% polymorphic (1 out of the 5 loci scored was monomorphic). Table 10 provides the percentage of polymorphic loci for each island population.

Private alleles were identified in 4 islands: Iraklia, Kopries, Naxos and Ovriokastro. Private alleles, by definition, are alleles that are unique or specific to a single population (Hartl and Clark, 1997) and can be taken as an indication of either a large population or an old population where a novel mutation has arisen. Iraklia and Naxos are both relatively large (> 448 Km^2). Although Ovriokastro and Kopries are relatively small (< 0.22 Km^2), Kopries is one of the oldest islands included in this study (11,700 years old). The number of private alleles found in each island population is given in Table 10.

A, the average number of alleles varied from 2.8 (Phtira and Andreas) to 12.8 (Naxos). It is not surprising that the large island of Naxos contained the largest number of alleles whereas Phtira, relatively large in area, appears to be the only surviving relict of a more widespread distribution of this lizard on the island of Paros. The average number of alleles (A) for each island population is given in Table 10.

Table 10: The number of individuals genotyped (N), the percentage of polymorphic loci (Loci), the number of private alleles (Pr. Alleles), the average number of alleles (A), average number of alleles divided by the range in allele size (M).

Island	N	Loci	Pr. Alleles	A	M
Agrilou	25	100	0	4.2	0.40
Andreas	17	100	0	2.8	0.44
Antikeros	19	100	0	6.2	0.37
Daskalio	30	100	0	6.0	0.48
Glaronissi	26	100	0	4.8	0.40
Phtira	19	40	0	2.8	0.26
Iraklia	17	100	5	9.4	0.38
Keros	13	80	0	6.4	0.27
Kopries	27	100	0	4.2	0.29
Koufonissi	16	100	0	4.4	0.46
Makronissi	24	80	2	4.8	0.47
Megalos Ambelas	24	100	0	2.4	0.32
Megali Plaka	25	80	0	4.0	0.26
Naxos	30	100	4	12.6	0.43
Ovriokastro	25	80	1	6.2	0.28
Schoinoussa	21	100	0	8.0	0.50

Bottleneck theory predicts that the reduced allele number relative to allele range (M) should persist longer than heterozygozity excess after a bottleneck event (Garza and Williamson 2001). Fixation of an allele results in an M value of zero, while a large, stable population, is expected to have an M value of 1.0 (Garza and Williamson 2001). Populations with small M values were: Phtira, Megali Plaka, Keros and Ovriokastro, suggesting that these populations may have experienced a severe population bottleneck. Not surprisingly, the islands of Naxos and Schoinoussa had the largest M values (> 0.50) suggesting that they are demographically stable. The M value for each island population is given in Table 10.

Not all loci behaved the same way, due to variation in the number and extent of alleles within each locus. For instance, the locus Pod1a has only four alleles total in the Cyclades, all of which are within a very small size range, with the most common genotype being 135 and 137. Due to the way in which M is calculated, any individual with this genotype will receive a score of 1.0, the highest possible value, because the distribution of alleles (2) covers the maximum extent of the range (2-8 bp depending on the island). This may have resulted in an inflated average M value for several islands.

Allele frequency shifts

The islands of Naxos, Iraklia, Schoinoussa and Antikeros (Figure 9), show the pattern predicted for larger populations that have not experienced a bottleneck. Their histograms show no modal shift, with many alleles within low frequency categories.

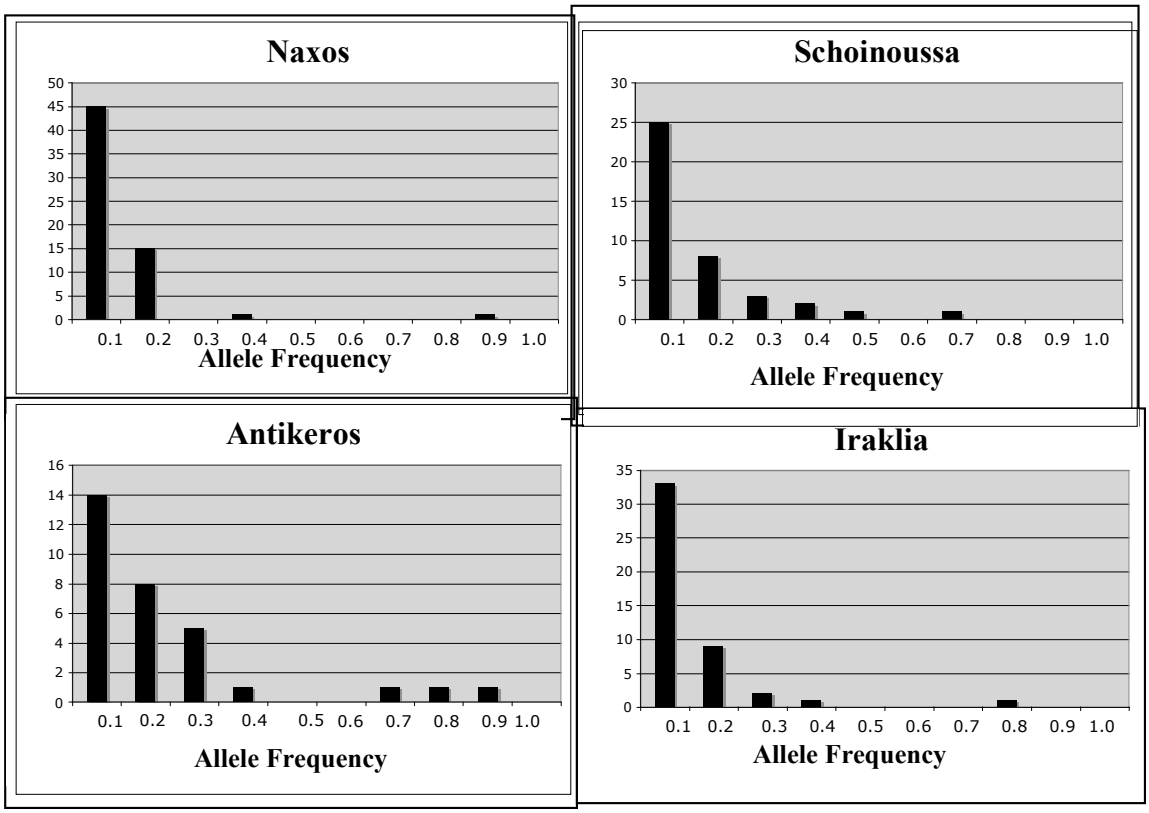

Figure 9: Histograms displaying the number of alleles within each frequency class. The number of alleles is given on the y-axis and the allele frequency classes are given on the x-axis. Allelic frequency classes were determined following the suggestion of Luikart (1998) and consisted of the islands of Naxos, Schoinoussa, Antikeros, and Iraklia have allelic frequency distributions consistent with a large panmictic population with a large number of rare alleles.

However, shifts in allele frequencies were observed in several islands. Glaronissi, Agrilou, Koufonissi, Daskalio, Andreas, Kopries, and Megalos Ambelas all showed the signature of a past bottleneck event (Figure 10) with an increase in the number of high frequency alleles.

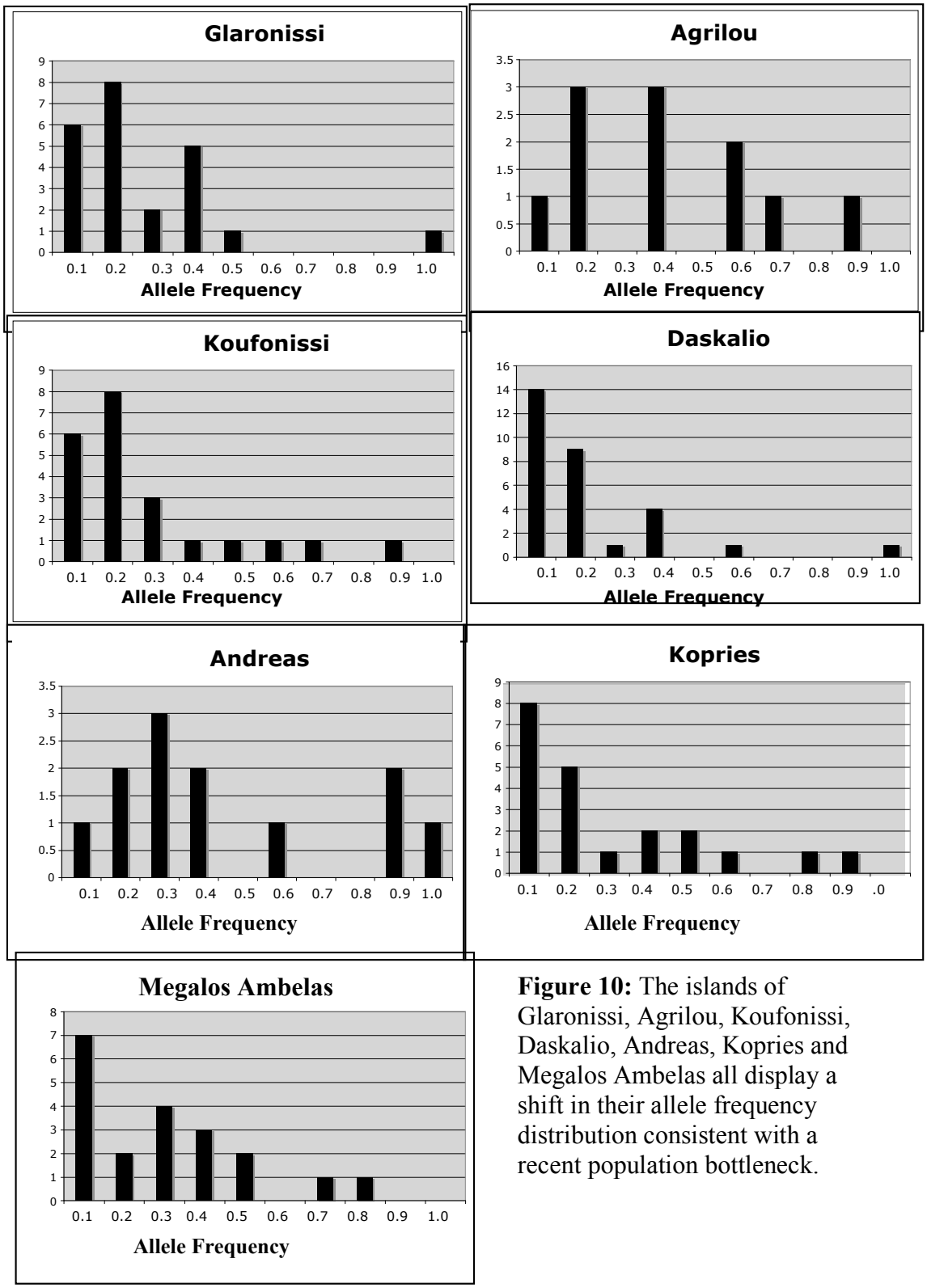

Figure 10: The islands of Glaronissi, Agrilou, Koufonissi, Daskalio, Andreas, Kopries and Megalos Ambelas all display a shift in their allele frequency distribution consistent with a recent population bottleneck.

Levels of Inbreeding

The inbreeding coefficient *f* (Weir and Cockerham, 1984) for each locus and across all loci is given by island in Table 11. A multiple linear regression analysis was conducted to see of the island explanatory variables: age, area or age*area explained a significant amount of the variance observed in *f*. None of these variables were significant (see Table 12). The interaction term was not significant and was removed from the model. Additionally *f* was used as an explanatory variable to see if it could significantly explain any of the parasite variables (tick prevalence, mite prevalence or total parasite burden). None of these regressions found a significant relationship between *f* and any of the parasite variables.

Table 11: Weir and Cockerham's *f* for each locus in every population and averaged among all loci in each population and among each locus and every population.

Island	**Lv319**	**Pb10**	**T434**	**Pod1a**	**Pod8**	**Avg. *f***
Andreas	+0.482	+0.328	+0.543	+0.055	- 0.143	0.257
Agrilou	- 0.228	+0.107	+0.527	+0.009	+0.380	0.159
Antikeros	+0.276	+0.033	+0.226	- 0.198	- 0.192	0.028
Daskalio	- 0.098	+0.116	+0.161	+0.018	- 0.036	0.032
Phtira	NA	- 0.025	NA	NA	+0.495	0.235
Glaronissi	- 0.014	- 0.037	+0.474	+0.220	+0.194	0.230
Iraklia	- 0.111	+0.191	+0.333	- 0.109	+0.025	0.098
Kopries	+0.025	+0.144	+0.537	+0.131	-0.182	0.182
Keros	- 0.152	- 0.106	+0.909	+0.161	NA	0.266
Koufonissi	+0.094	+0.067	+1.000	+0.023	+0.017	0.166
Makronissi	- 0.057	- 0.163	+0.545	+0.018	NA	0.118
Megaos Ambulas	- 0.187	+0.149	+0.413	+0.069	+0.020	0.033
Megali Plaka	+0.093	NA	+1.000	-0.215	+0.138	0.209
Naxos	- 0.053	+0.166	+0.404	- 0.188	- 0.043	0.169
Nea Kameni	+0.014	- 0.020	+0.418	+0.129	+0.167	0.084
Ovriokastro	+0.732	+0.071	+0.500	+0.176	NA	0.370
Schoinoussa	+0.144	+0.071	+0.487	+0.052	- 0.206	0.198
Avg. *f* per locus	+0.06	+0.07	+0.499	+0.020	+0.05	

Multiple linear regression analyses were carried out with the explanatory variables: age, area and age*area and each of the following explanatory variables: heterozygozity (He), allelic richness (A), and M. The interaction term was not significant and was subsequently removed from the analysis. Type III sum of squares as well as the p and R^2 values for the entire model and the p value for each of the explanatory variables is available in Table 9. The only genetic variable that had a significant relationship to any of the explanatory variables was allelic richness. There was a significant relationship observed between log(area) and allele number ($p = 0.0202$) suggesting that the larger the area, the more genetic variation is maintained. A significant relationship was also observed between log(area) and

heterozygozity (p = 0.0076). This conforms well with our prediction that island area would be positively correlated with genetic diversity and suggests that larger islands support a larger population size and greater number of alleles than smaller islands, as predicted by Brown (1971). Additionally there was a noticeable but non-significant negative relationship (p = 0.1516) observed between allele number and age in the multiple regression model, suggesting that the older the island the less genetic variation will be maintained. The age*area interaction term was not significant and was subsequently removed from the model. For each relationship a single linear regression was also conducted removing the other explanatory variable, the p and R^2 values are given in Table 12. Figure 11a shows the linear regression of average number of alleles (A) plotted against log(Area) whereas Figure 11b shows the regression of expected heterozygozity (He) plotted against island log(area).

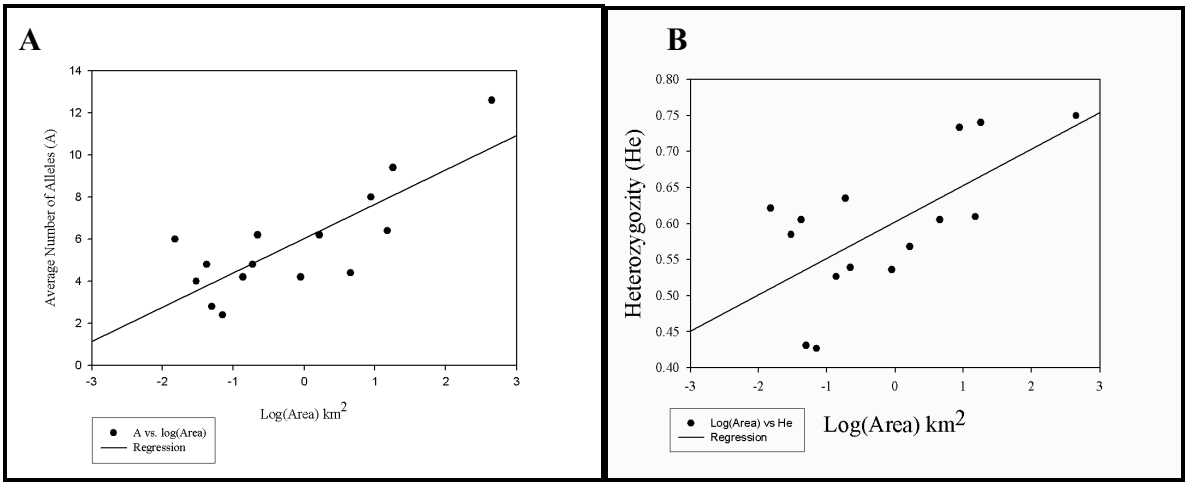

Figure 11: A) Scatter plot of A, average number of alleles plotted against log(Area), the linear regression between these variables is given by a straight line. B) Scatter plot of He, heterozygozity vs. log(Area), the linear regression is given by a straight line.

Multiple linear regression analyses were also carried out to examine how the explanatory variables: age, log(area) and age*log(area) may explain parasite abundance and prevalence (mite prevalence, tick prevalence and total parasite burden). In all cases, the interaction term was not significant and was subsequently removed from the analysis. Table 12 contains the p and R^2 values for the entire model and the p value for each of the explanatory variables. There was a significant relationship observed between island age and mite prevalence (p = 0.0374 and R^2 = 0.5098). Figure 12a displays Mite Prevalence plotted against island age along with the regression line for the analysis. Additionally there was a significant relationship observed between total parasite burden and age (p = 0.0168), although a significant relationship was not observed between tick prevalence and island age (p = 0.3871). Figure 12b displays total burden plotted against island age (years), along with the regression line for the analysis.

Island area did not explain a significant amount of the variance observed for either measure of parasite prevalence or abundance.

Figure 12: A) Scatter plot of mite prevalence, plotted against island age (years), the linear regression between these variables is given by a straight line. B) Scatter plot of total parasite burden plotted against island age (years), the linear regression is given by a straight line.

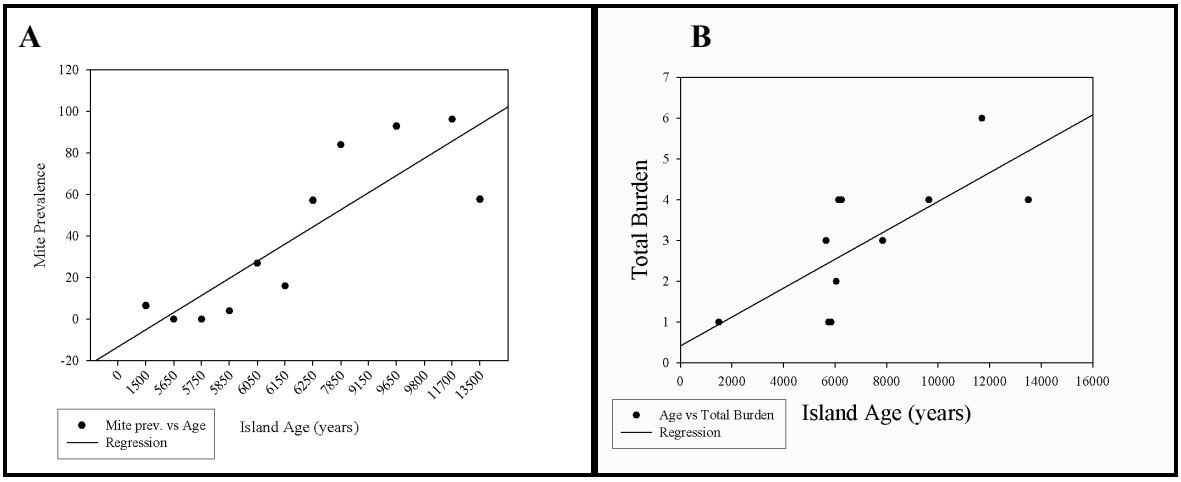

Table 12: General Linear Models (GLM) island variables on genetic and parasite measures.

Whole Model:	R^2	P	P values
Dependent variable: A	0.778	**0.0006**	
Age			0.0617
Log(Area)			**0.0002**
Dependent variable: He	0.497	**0.0160**	
Age			0.2627
Log(Area)			**0.0076**
Dependent variable: M	0.031	0.8278	
Age			0.6885
Log(Area)			0.6609
Dependent variable: *f*	0.028	0.8348	
Age			0.6520
Log(Area)			0.7142
Dependent variable: Mite Prevalence	0.682	0.0577	
Age			**0.0374**
Log(Area)			0.9265
Dependent variable: Tick Prevalence	0.14635	0.5310	
Age			0.3871
Log(Area)			0.4841
Dependent variable: Total Burden	0.5387	**0.0453**	
Age			**0.0431**
Log(Area)			0.6146

Simple linear regression analyses were also carried out to the explanatory parasite variables: mite prevalence, tick prevalence and total parasite burden, using measures of genetic diversity as the predictor variable. There was no significant relationship observed between any of these variables. Table 13 contains all of the p and R^2 values for each of these regression analyses.

Table 13: Simple Linear Regression

	R^2	P value
Dependent variable: A		
Age	0.0998	0.2515
Log(Area)	0.6347	**0.0004**
Dependent variable: He		
Log(Area)	0.4400	**0.0070**
Dependent variable: Mite Prevalence		
Age	0.5093	**0.0137**
Allelic richness (A)	0.0036	0.8607
Heterozygozity (He)	0.0000	0.9978
M (k/r)	0.2761	0.0969
f (inbreeding)	0.1239	0.2884
Grazing history (score)	0.0033	0.8675
Dependent variable: Tick Prevalence		
Allelic richness (A)	0.0068	0.8097
Heterozygozity (He)	0.0009	0.9288
M (k/r)	0.0813	0.3954
f (inbreeding)	0.1200	0.2966
Grazing history (score)	0.7340	**0.0008**
Dependent variable: Total Burden		
Age	0.5229	**0.0119**
Allelic richness (A)	0.0098	0.7727
Heterozygozity (He)	0.0060	0.8208
M (k/r)	0.2665	0.1040
f (inbreeding)	0.0013	0.9175
Grazing history (score)	0.3282	0.0655

When each of the parasite variables (Mite Prevalence, Tick Prevalence and Total Parasite Burden) were regressed against grazing severity (measured as grazing score, see Table 1) a significant relationship ($p = 0.002$ and $R^2 = 0.792$) was observed between tick prevalence and grazing severity. However, neither of the other variables: mite prevalence or total parasite burden were significant. Figure 13 displays a plot of tick prevalence against the grazing score, with the regression given by a straight line.

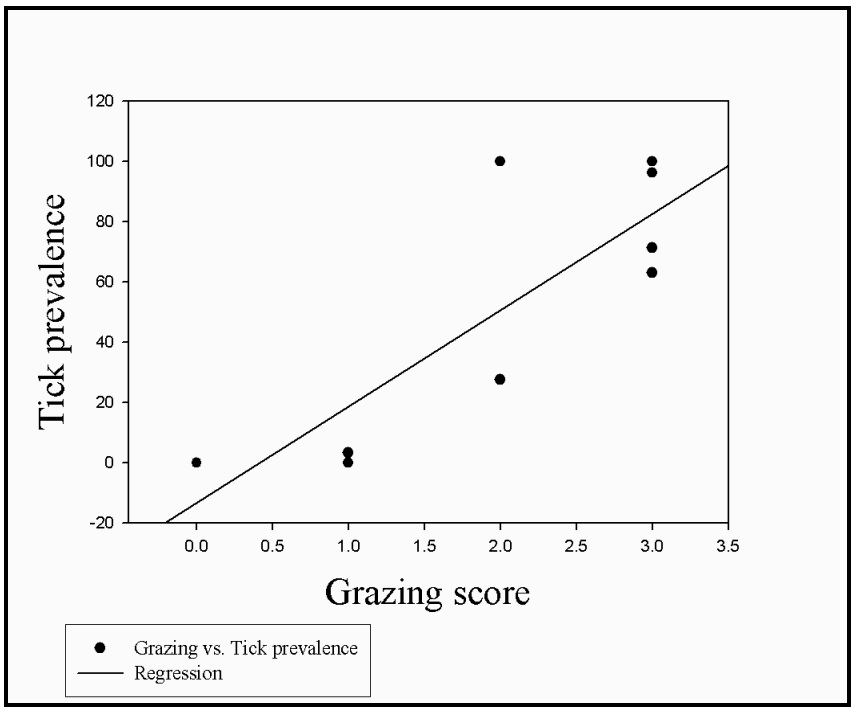

Figure 13: Scatter plot of tick prevalence plotted against grazing score, the linear regression between these variables is given by a straight line.

Isolation by Distance:

Microsatellite IBD (Distance between islands)

The relationship between genetic distance (F_{ST}) and geographic distance was positive but not significant ($p = 0.3301$; Figure 14a). Strangely when the outlier islands of Phtira and Nea Kameni were excluded from the analysis, a negative relationship was observed between genetic and geographic distance, but was also not significant ($p = 0.7310$; Figure 14b). This aligns well with our predictions that there would be no isolation by distance pattern as a result of no gene flow between islands.

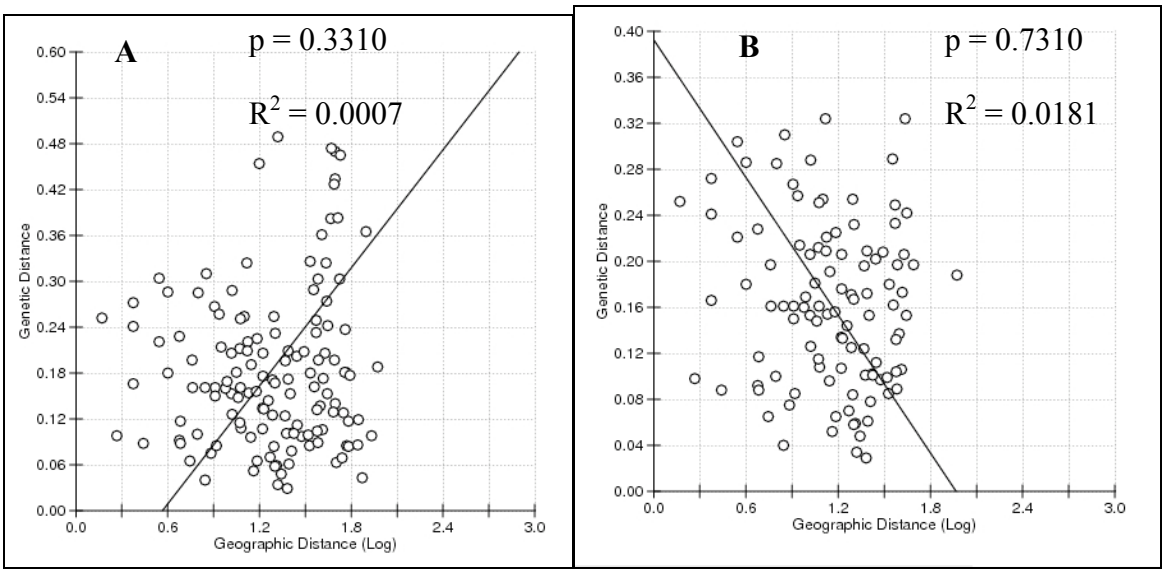

Figure 14: Mantel test, F_{st} was used as a measure of genetic distance (y-axis) and was plotted against the distance (Km) between islands. Figure 14A includes all islands, while Figure 14B excludes the outliers Phtira and Nea Kameni.

Microsatellite IBD (Depth between islands)

The relationship between genetic distance (F_{ST}) and maximum island depth was not significant (p = 0.44 and R2 = 0.0001; Figure 15a). When the outlier islands of Phtira and Nea Kameni were excluded from the analysis (Figure 15b), a positive relationship was observed between genetic distance and maximum island depth. This was also not significant (p = 0.253 and R^2 = 0.01) indicating that there is no relationship between these variables.

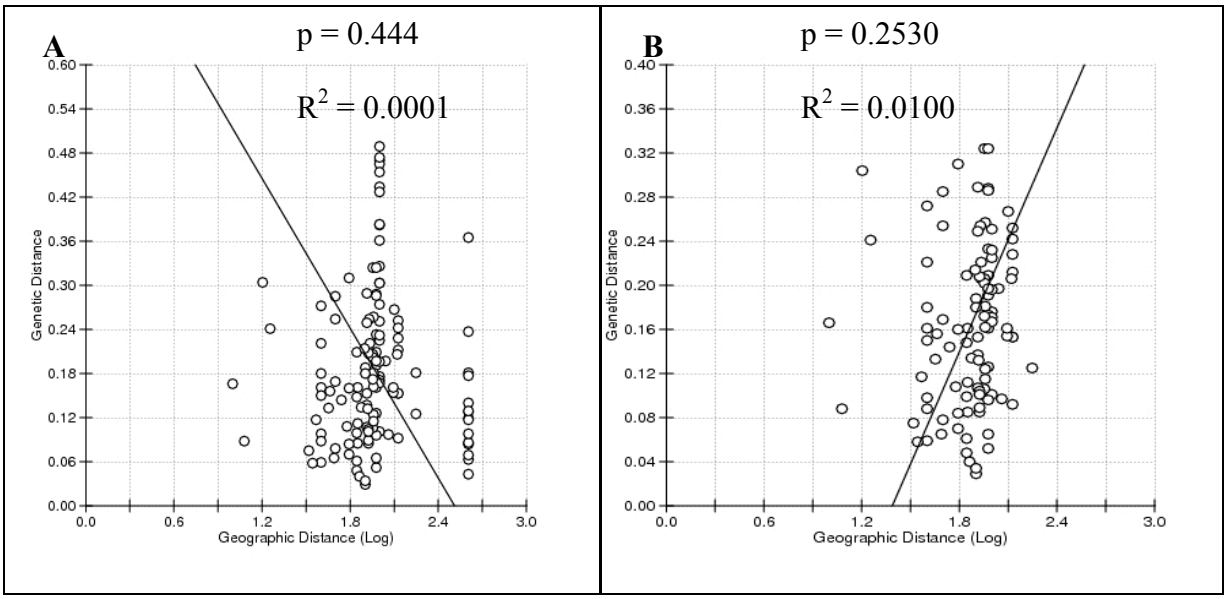

Figure 15: Mantel Test, Fst was used as a measure of genetic distance (y-axis) and was plotted against the maximum log(depth) in meters separating islands. Figure 15A contains all islands and Figure 15B excludes the outliers Phtira and Nea Kameni.

DISCUSSION

The continental land fragments described in the present study provide an unrivalled opportunity to test the effects of island age, area and isolation on genetic diversity of island biota. The widespread prevalence and weak dispersal abilities of *P. erhardii* also make this species an ideal study organism for testing predictions about how island characteristics may influence genetic variation and evolutionary potential.

Although genetic diversity at the mitochondrial cytochrome locus was low, these data suggest that the limited amount of genetic variation on many islands was likely a product of bottleneck history resulting from the subsequent separation of island populations due to sea level rises. Although haplotype and nucleotide diversity was high on large islands like Naxos, most of the smaller islands in this study had only a few haplotypes or had drifted completely to fixation. This suggests that island populations have experienced very little to no gene flow since island inception and that genetic drift is the driving force, causing the extinction of haplotypes and impoverished levels of haplotypic diversity.

Phylogenetic analysis of cytochrome b recovered the two previously described lineages of *P. erhardii* (Poulakakis et al. 2003) and provides weak support for several regionally defined haplogroups. However, many of these haplotypes are not confined to a single haplogroup, suggesting either fixation of shared ancestral polymorphism, extensive gene flow between islands or both. The haplotype network is more suitable to portraying population genetic variation and indicates that associations among mitochondrial haplotypes are consistent with a pattern of historical fragmentation of a formally contiguous landmass, with the same haplotypes occurring across islands that were once part of the same mother island.

Islands that share a common history (ie. were derived from the same landmass) possess closely related haplotypes with two notable exceptions: Phtira and the Nea Kameni/Santorini complex. Phtira is highly divergent from other haplotypes within the region despite its proximity and shared history with Naxos and surrounding islets (Van Andel & Shackleton, 1982). This genetically distinct population may have survived as a relic of the ancestral genetic diversity present on the island of Paros. Interestingly, the large island of Paros immediately adjacent to Phtira is completely devoid of *P. erhardii*. It has been suggested that its absence could be due in part to a past disease or epidemic that resulted in the extinction of *P. erhardii* on this island (Foufopoulos pers. comm.). Judging from the mitochondrial phylogeny, the island of Amorgos also shared a recent common ancestor with the other islands of the Central Cyclades,

but diverged more recently than Phtira. According to Dermitzakis (1987), Amorgos has not been connected to the Cyclades since the Pliocene. Thus the Phtira haplotype may have survived as a relic of an ancestral haplotype present in the Cyclades prior to this time.

The other atypical haplotype distribution is that of the Nea Kameni/Santorini complex, which groups both with the Schoinoussa cluster and with other sequences found more than 50 km south in Santorini, Nea Kameni and Anafi. This second haplogroup of Nea Kameni/Santorini is quite divergent from the haplotypes found within the Schoinoussa cluster, with 19 or more mutational changes separating them. One potential explanation for the patterns of haplotype distribution observed in Nea Kameni/Santorini and Schoinoussa is that of long distance colonization. If we are to acknowledge the possibility of long distance dispersal among islands, we must address the prevalence of these dispersal events and reconcile the direction and magnitude of such events with the distribution of haplotypes that we have observed on the islands examined. There are several characteristics of Nea Kameni that make it an ideal target for colonization events. First, it is a very young volcanic island that was previously uninhabited, so that any migrants will not have to compete with a resident population to become established. There is high traffic from Santorini to Nea Kameni, and as Santorini surrounds Nea Kameni on all sides it is not surprising that some of the haplotypes found on Nea Kameni come from Santorini. Second, the island complex of Santorini and Nea Kameni is one of the most sought after tourist destinations in the Cyclades, with a large number of boats arriving or departing from these islands each day, thereby providing ample opportunities for a long-distance colonization event to and from ports such as Schoinoussa. With the exception of the Nea Kameni/Santorini complex we have not detected any other long distance dispersal events. The high amount of nucleotide sequence diversity observed on the Nea Kameni/Santorini complex is far greater than any other island examined and could also be indicative of colonization events from other islands throughout the Aegean. However, since our sampling was not exhaustive we were only able to determine one source population responsible for the genetic make up of the population of Nea Kameni. Also, there was similar haplotype sharing among the Nea Kameni/Santorini complex with both the western island of Anafi and the island of Antikythira, which could either be due to a shared ancestral haplotypes or evidence of yet another dispersal/colonization event.

Tests conducted for isolation by distance (Mantel Test) found no relationship between genetic distance and either the straight-line distance separating islands or the depth of the underwater saddle separating islands. This is not surprising when we consider the findings of Bittkau and Comes (2005), who also found no relationship between geographical distance and measures of genetic distance (F_{ST}) in

their *Nigella* cpDNA from the Cyclades. The pattern observed in the present study corresponds well with the Case III model described by Hutchinson and Templeton (1999) and is indicative of drift being more influential than gene flow.

Microsatellites however provide a more sensitive measure of fragmentation history. Results show a strong positive area effect. In contrast, age has a non-significant but discernibly negative effect on allelic richness. This supports our previously outlined predictions that island area would be positively associated with genetic diversity, with smaller islands maintaining less genetic variation than larger islands. Although a non-significant negative relationship between age and genetic diversity was detected, it is indicative of the proposed cumulative effects of drift on smaller, older islands. While these findings are not particularly surprising, it is noteworthy that allelic richness (A) and heterozygozity (He) were the only two measures of genetic diversity that were significantly described by an island characteristic. Of the two, allelic richness has a stronger relationship with island area than did heterozygozity. It has been shown both theoretically and experimentally that allelic richness is a much more sensitive indicator of bottleneck events than heterozygozity, lending considerable support to the observations reported here (Leberg, 2002).

A shift in the mode of the allele frequency distribution was also detected on several islands, suggesting a past bottleneck event. In contrast, the larger islands of Naxos, Schoinoussa, Iraklia and Antikeros did not show the same signatures typical of a historical bottleneck. This is interesting when we consider that these are four out of the five largest islands included in this study. Excluding islands that did not meet the minimum requirement for the test (five polymorphic loci), signatures of past bottleneck effects were observed in all smaller islands. Several of these islands (Phtira, Antikeros, Makronissi, Megali Plaka and Ovriokastro) also have low numbers of polymorphic loci, consistent with their bottleneck history.

Only four islands contained private alleles, Iraklia, Makronissi, Naxos and Ovriokastro. Private alleles can be due to either the retention of rare alleles from the ancestral population, or novel alleles that have arisen from mutation. Both Iraklia and Naxos are relatively large compared to the other islands in the study (18.078 and 448 km^2 respectively), and could contain rare alleles that have been lost due to drift in other smaller islands. Both Makronissi and Ovriokastro are of a moderate age (5850 and 5750 years respectively) and medium size (0.042 and 0.22 km^2 respectively), making it difficult to speculate as to whether their private alleles are due to the retention of rare (ancestral) alleles or novel (mutated) alleles. The presence of private alleles is also indicative of a lack of gene flow between populations. While we

expect there to be little to no gene flow between most of the islands in the Cyclades (with the notable exception of the Nea Kameni/Santorini complex), the distribution of private alleles could also reflect the fragmentation history of these islands. It is also possible that the private alleles found on Iraklia, Makronissi and Ovriokastro do occur on the large mother island of Naxos but were not sampled. While most of the islands included in this study share a common isolation history, either with the Naxos, the Iraklia, or the Keros Cluster, not every island once part of these island complexes was sampled. For example the relatively large island of Iraklia has several private alleles not shared with any of the other islands included in this study, however the islands adjacent to Iraklia: Agrilos and Fidoussa, were not sampled.

The parameter (M) does not appear to follow the predicted outcome of population bottleneck theory with more recently isolated islands. Another study conducted by Whitehouse and Harley (2001) used microsatellite variation to assess the effects of a known population bottleneck on African elephants. In this study, the authors also failed to find a reduction in M. The authors offer two potential causes for this result: 1) that the deviation could be due a non-stepwise mutation (mutation events favoring the increase or decrease or alleles by more than one repeat unit instead of only a single step), or 2) it could be the result of a restricted range in allele size. We also observed a restriction in allele range in our data set, especially for Pod1a, which has maximal range of only 8 base pairs. Limited allele range would tend to bias M because a loss in largest or smallest allele becomes more likely, and if either is lost, the range contracts, thus obscuring any reduction in M and consequently any detection of a bottleneck effect. This may also help to explain M was not significantly explained by island log(area) while both of the other genetic variables allelic richness (A) and heterozygozity (He) were.

With respect to parasite burden, island age was the only significant predictor of both mite prevalence and consequently total parasite burden. However ticks had no relationship suggesting that total parasite burden is driven entirely by mites. This is an interesting finding, since the role of population bottlenecks and inbreeding on parasite prevalence is a subject of much discussion. While parasite prevalence has been previously linked to impoverished levels of genetic diversity in some instances (Coltman et al. 1999), it is not so in other studies (Hale & Briskie 2007; Stevens et al. 1997). This could be due to the host breeding condition, the season, the stress the host is experiencing, all of which may also affect ectoparasite loads (Hawley 2007; Tompkins 2007). This is particularly interesting when we consider the fact that none of the genetic variation indices measured in this study (M, A, *f* and He) were able to significantly explain the amount of parasite prevalence observed in the island populations. While there was not a significant effect of inbreeding or any other genetic measure on parasite abundance it is

important to consider that the weak loss in genetic diversity with island age may still be substantial enough to compromise parasite resistance and requires further study. Conceivably a reduction in genetic diversity following a population bottleneck, such as the one experienced by a population following island formation, could result in a reduction in genetic variability. It has been shown that inbred individuals are often less capable of responding to immunocompetence challenges. Because blood feeding parasites such as mites directly encounter the immune systems of their host, therefore individuals from older islands might be more susceptible to parasites. Haematophagous parasites have been shown to exert fitness costs on their hosts such as a reduction in clutch size (Wiehn et al. 1999), body condition and cause the transmission of vector borne pathogens (Wakelin, 1996).

Perhaps of greater interest is the association between grazing intensity and parasite burden. The linear regression models using the predictor variable tick prevalence against grazing score, yielded a positive significant relationship. It has been shown previously that land alteration and have an affect on the transmission rate and frequency of tick borne diseases (for review see Hoogstraal 1981). Additionally host switching from domestic livestock to wild animals has been documented (Gonzales-Acuna et al. 2004). The observed relationship may result from host switching following the introduction of livestock to the island. However, simple linear regression of the other parasite predictor variables (mite prevalence and total parasite burden) against the grazing score was not significant. Additionally, grazing practices could impact the island populations in several ways; by fragmenting suitable habitat, changing transmission dynamics and increasing the amount of stress experienced by the hosts. An earlier study demonstrated that some lizards show no avoidance of parasitized con-specifics suggesting that parasite loads may actually decrease in clumped species distributions (Sorci et al. 1997), and may not change the transmission dynamics. Further work needs to be done to assess parasite diversity on these islands to determine if parasites were introduced recently or might be co-evolving with their host. Additionally, further work should be done to test the effects of island history on other types of parasites, especially those that may cause a more substantial fitness cost in the blood or gut.

This study has addressed the role of island variables on the persistence of genetic diversity and found a significant area affect. This is important finding for conservation biologists, when considering the most important variables to include in reserve design in order to maintain adequate levels of genetic diversity. We have also established that there is a significant effect of age on mite abundance. This finding merits further investigation since there is still much debate surrounding the role of inbreeding and diminished genetic diversity on parasite prevalence in natural populations. Future work is planned to address the relationship between genetic diversity and disease resistance in these island reptile

populations in order to gauge the potential fitness consequences of loss in genetic variability. Additionally we have established that grazing history is a significant indicator of tick prevalence on islands. This finding may provide an example of host switching in a natural population, causing a shift in parasite communities on islands due to domestic animal practices. This in turn could have implications for conservation and introduction of exotic diseases. Further work needs to be conducted in order to address the extent in which grazing practices have altered native island parasite fauna.

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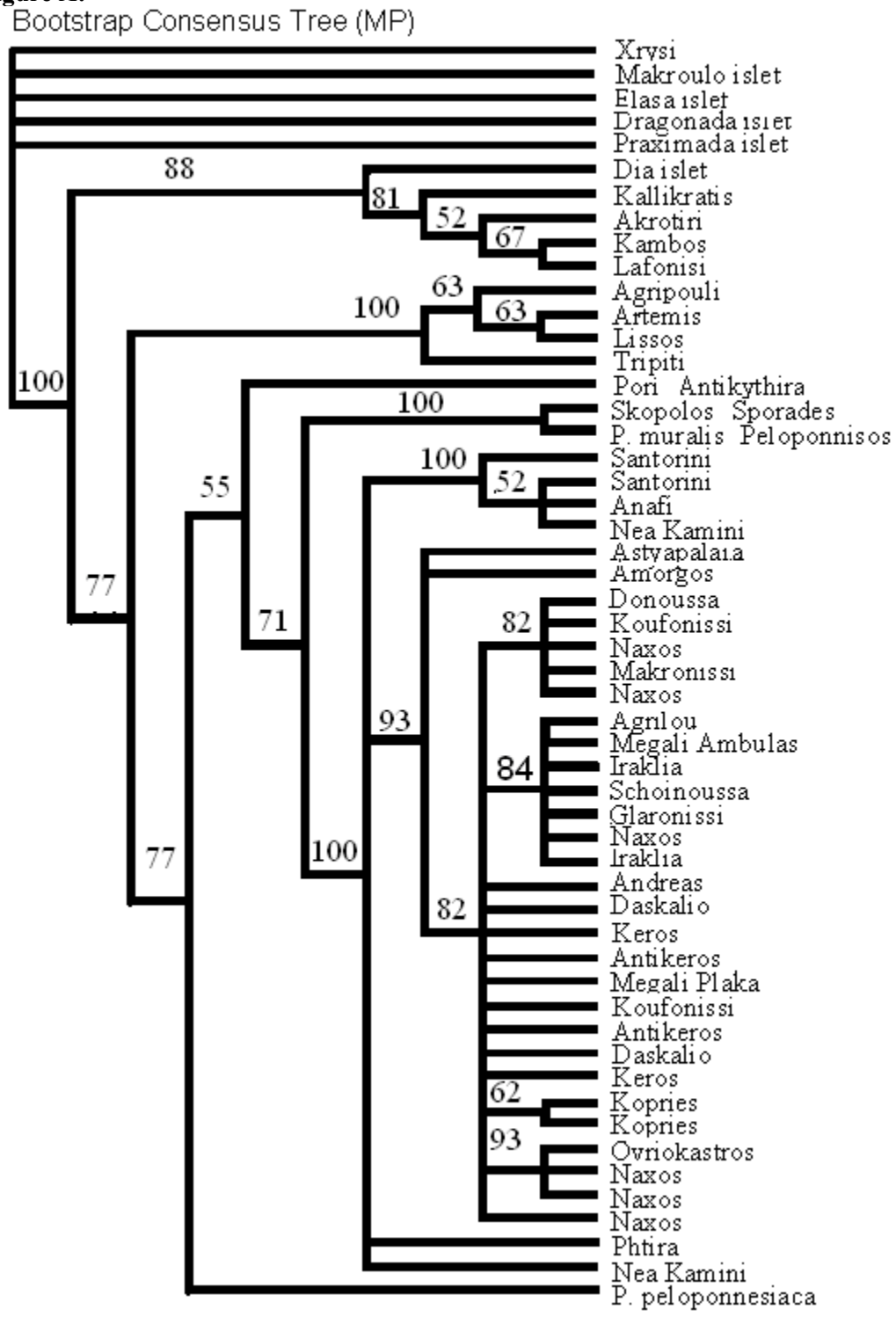
APPENDIX

Figure A:



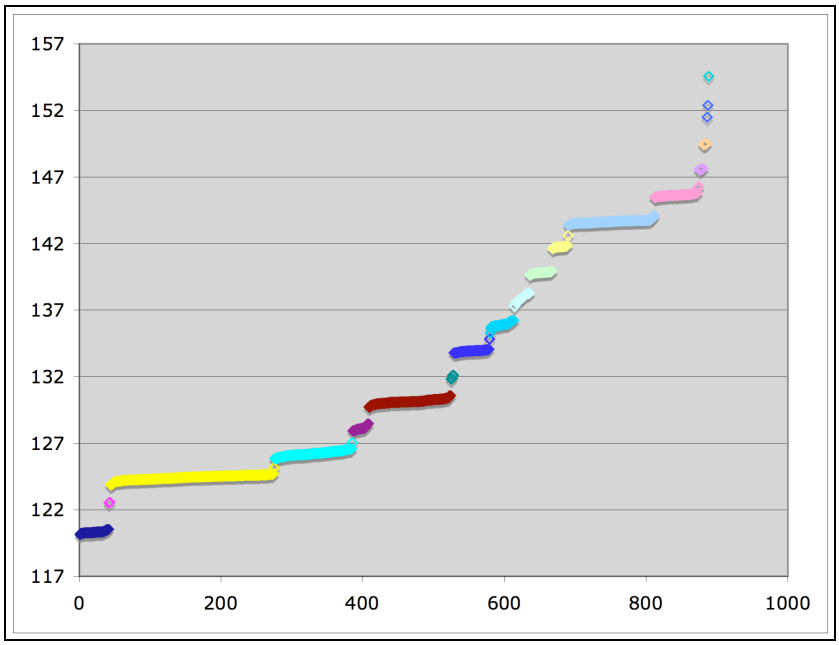


Figure B: The binning results for Lv319 generated using the Flexibin program. Allele size in base pairs is given on the y-axis, the x-axis gives the number of each allele, alleles were sorted by size prior to binning to aid in determining the correct bins. Each allele is represented by a different color for clarity.

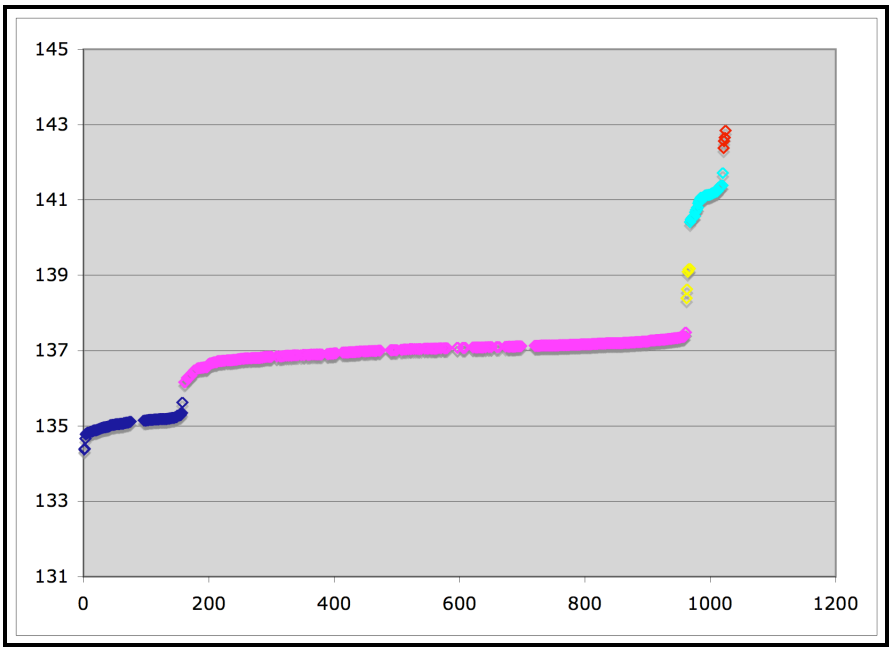


Figure C: The binning results for Pod1a generated using the Flexibin program. Allele size in base pairs is given on the y-axis, the x-axis gives the number of each allele, alleles were sorted by size prior to binning to aid in determining the correct bins. Each allele is represented by a different color for clarity.

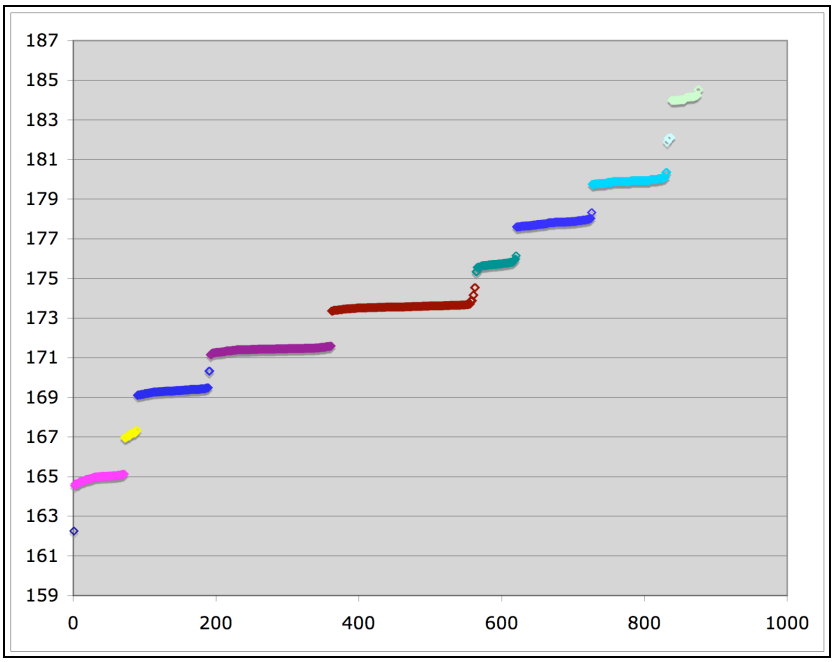


Figure D: The binning results for T434 generated using the Flexibin program. Allele size in base pairs is given on the y-axis, the x-axis gives the number of each allele, alleles were sorted by size prior to binning to aid in determining the correct bins. Each allele is represented by a different color for clarity.

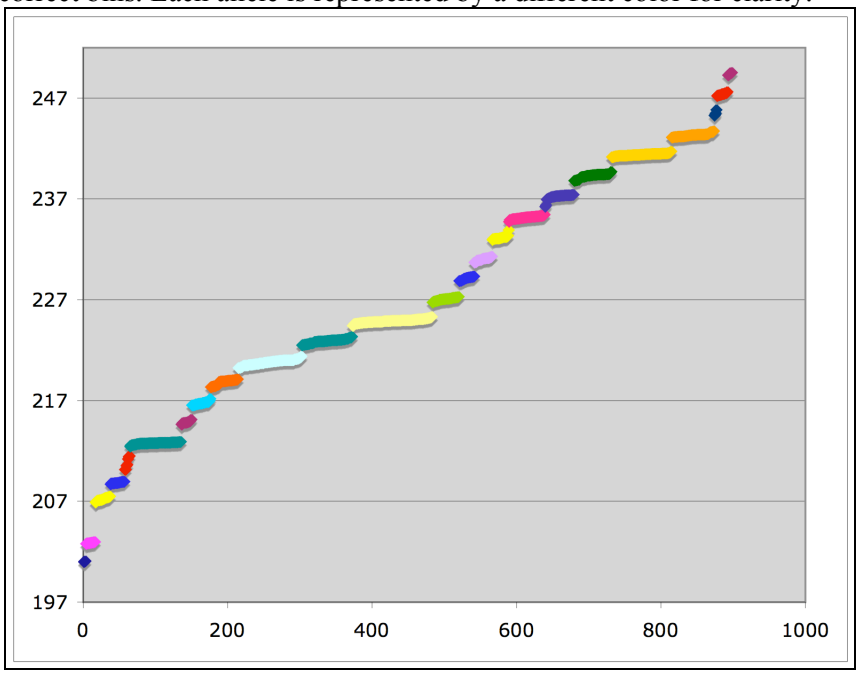


Figure E: The binning results for Pb10 generated using the Flexibin program. Allele size in base pairs is given on the y-axis, the x-axis gives the number of each allele, alleles were sorted by size prior to binning to aid in determining the correct bins. Each allele is represented by a different color for clarity.

Table A: Lv319

Repeats	Length	Mean size (bp)	Std. Dev.	Count
1	120.44	120.33	0.079	41
5	128.28	128.13	0.144	23
6	130.14	130.15	0.156	116
7	132.08	138.97	0.149	4
8	134.02	133.96	0.140	51
11	139.84	139.82	0.068	32
12	141.78	141.80	0.185	23
13	143.72	143.66	0.111	121
14	145.66	145.66	0.127	63
15	147.60	146.59	0.043	6
16	149.54	149.47	0.027	5

Table B: Pod 1a

Repeats	Length	Mean size (bp)	Std. Dev	Count
1	135.06	135.08	0.157	158
2	137.08	137.01	0.210	803
3	138.98	138.94	0.309	6
4	140.94	141.04	0.284	53
5	142.90	142.61	0.192	4

Table C: Pb10

Repeats	Length	Mean size (bp)	Std. Dev	Count
1	200.52	201.03	0.056	3
2	202.56	202.90	0.053	13
4	206.64	206.23	0.170	21
5	208.68	208.87	0.072	20
6	210.72	210.33	0.199	4
6.5*	211.77	211.38	0.122	3
7	212.76	212.79	0.096	71
8	214.80	214.86	0.134	15
9	216.84	216.75	0.149	26
10	218.88	218.83	0.253	38
11	220.92	220.81	0.258	88
12	222.96	222.91	0.201	70
13	225.00	224.92	0.168	111
14	227.04	227.08	0.146	37
15	229.08	229.13	0.151	21
16	231.12	231.04	0.172	25
17	233.15	233.19	0.179	23
18	235.20	235.18	0.144	50
19	237.24	237.25	0.249	40
20	239.28	239.31	0.214	52
21	241.32	241.42	0.110	83
22	243.36	243.35	0.149	59
23	245.40	245.50	0.235	4
24	247.44	247.40	0.105	15
25	249.48	249.42	0.111	6

Table D: T434

Repeats	Length	Mean size (bp)	Std. Dev	Count
1	165.08	164.94	0.143	70
2	167.20	167.13	0.116	18
3	169.32	169.35	0.166	102
4	171.44	171.43	0.081	170
5	173.56	173.59	0.138	202
6	175.68	175.72	0.125	57
7	177.80	177.81	0.123	106
8	179.92	172.99	0.084	105
9	182.04	182.06	0.129	6
10	184.16	184.11	0.129	39

Table E: HW deviations for each locus in every population

Population	**Locus**	**Observed Heterozygozity**	**Expected Heterozygozity**	**P-value**	**Standard Deviation**
Andreas	Lv319	0.04762	0.09408	0.02625	0.00046
Andreas	Pb10	0.42857	0.63298	0.08204	0.00084
Andreas	T434	0.26087	0.56425	0.00016***	0.00004
Andreas	Pod8	0.30435	0.32174	1.0	0.00000
Andreas	Pod1a	0.28000	0.24571	1.0	0.00000
Agrilou	Lv319	0.73333	0.59944	0.54244	0.00163
Agrilou	Pb10	0.70000	0.78249	0.09518	0.00069
Agrilou	T434	0.10000	0.20960	0.03060*	0.00051
Agrilou	Pod8	0.70000	0.70621	0.74733	0.00138
Agrilou	Pod1a	0.26667	0.42712	0.07502	0.00083
Antikeros	Lv319	0.23529	0.32086	0.43834	0.00161
Antikeros	Pb10	0.89474	0.92461	0.67197	0.00069
Antikeros	T434	0.31579	0.40541	021050	0.00108
Antikeros	Pod8	1.00000	0.83926	0.96155	0.00057
Antikeros	Pod1a	0.52632	0.44381	0.60742	0.00156
Daskalio	Lv319	0.73333	0.66893	0.47305	0.00125
Daskalio	Pb10	0.73333	0.82825	0.27862	0.00070
Daskalio	T434	0.63333	0.75311	0.47756	0.00136
Daskalio	Pod8	0.70000	0.71243	0.20828	0.00086
Daskalio	Pod1a	0.10000	0.09661	1.00000	0.00000
Phtira	Lv319	Monomorphic			
Phtira	Pb10	0.47368	0.46230	1.00000	0.00000
Phtira	T434	Monomorphic			
Phtira	Pod8	Monomorphic			
Phtira	Pod1a	0.15789	0.30868	0.07621	0.0080
Glaronissi	Lv319	0.75000	0.74025	0.57648	0.00169

Glaronissi	Pb10	0.80000	0.77224	0.89140	0.00100
Glaronissi	T434	0.38462	0.72474	0.00022***	0.00005
Glaronissi	Pod8	0.56000	0.71510	0.07179	0.00068
Glaronissi	Pod1a	0.19231	0.23756	0.37575	0.00153
Iraklia					
	Lv319	0.94118	0.85027	0.86464	0.00077
Iraklia	Pb10	0.76471	0.93939	0.02343*	0.00022
Iraklia	T434	0.52941	0.78610	0.06538	0.00051
Iraklia	Pod8	1.00000	0.90524	0.72368	0.00080
Iraklia	Pod1a	0.35294	0.36185	0.55967	0.00126
Kopries	Lv319	0.70370	0.72117	0.51906	0.00165
Kopries	Pb10	0.33333	0.38854	0.24565	0.00103
Kopries	T434	0.22222	0.47519	0.00918**	0.00033
Kopries	Pod8	0.59259	0.67994	0.06951	0.00060
Kopries	Pod1a	0.33333	0.28302	1.00000	0.00000
Keros	Lv319	0.46154	0.40308	1.00000	0.0000
Keros	Pb10	1.00000	0.90769	1.00000	0.0000
Keros	T434	0.07692	0.81538	0.00000***	0.0000
Keros	Pod8	0.76923	0.91077	00.03685*	0.00033
Keros	Pod1a	Monomorphic			
Koufonissi	Lv319	0.50000	0.55040	0.26881	0.00113
Koufonissi	Pb10	0.75000	0.80242	0.14377	0.00099
Koufonissi	T434	0.00000	0.22581	0.00310**	0.00018
Koufonissi	Pod8	0.81250	0.83065	0.29071	0.00131
Koufonissi	Pod1a	0.50000	0.50806	1.00000	0.00000
Makronissi	Lv319	0.73913	0.71401	0.04542*	0.00062
Makronissi	Pb10	0.95238	0.82230	0.80628	0.00105
Makronissi	T434	0.33333	0.72429	0.00019***	0.00004
Makronissi	Pod8	0.76190	0.77584	0.85158	0.00115
Makronissi	Pod1a	Monomorphic			
Megalos Ambelas	Lv319	0.73913	0.62512	0.61500	0.00136
Megalos Ambelas	Pb10	0.57143	0.66899	0.66409	0.00143
Megalos Ambelas	T434	0.20833	0.35195	0.00360**	0.00016
Megalos Ambelas	Pod8	0.75000	0.80408	0.33511	0.00132
Megalos Ambelas	Pod1a	0.45833	0.46720	1.00000	0.00000
Megali Plaka	Lv319	0.44000	0.48408	0.46347	0.00150
Megali Plaka	Pb10	Monomorphic			
Megali Plaka	T434	0	0.37224	0.000***	0.00000
Megali Plaka	Pod8	0.72000	0.59510	0.51525	0.00148
Megali Plaka	Pod1a	0.42308	0.50302	0.45186	0.00153
Naxos	Lv319	0.96429	0.91688	0.86154	0.00042
Naxos	Pb10	0.77778	0.92942	0.00000***	0.00000
Naxos	T434	0.50000	0.83277	0.00057***	0.00007

Naxos	Pod8	0.73333	0.90056	0.02081*	0.00029
Naxos	Pod1a	0.30000	0.26780	1.00000	0.00000
Nea Kameni	Lv319	0.80000	0.81130	0.54507	0.00065
Nea Kameni	Pb10	0.89286	0.87597	0.58871	0.00056
Nea Kameni	T434	0.40000	0.68249	0.00112**	0.00012
Nea Kameni	Pod8	0.73333	0.84011	0.06966	0.00055
Nea Kameni	Pod1a	0.25806	0.36224	0.19883	0.00139
Ovriokastro	Lv319	0.22727	0.71882	0.00000***	0.00000
Ovriokastro	Pb10	0.76190	0.81882	0.18344	0.00079
Ovriokastro	T434	0.31818	0.62896	0.00125**	0.00011
Ovriokastro	Pod8	0.52174	0.63092	0.04527*	0.00047
Ovriokastro	Pod1a	Monomorphic			
Schoinoussa	Lv319	0.71429	0.83159	0.05437	0.00070
Schoinoussa	Pb10	0.90000	0.91538	0.77882	0.00088
Schoinoussa	T434	0.38095	0.73403	0.00152**	0.00011
Schoinoussa	Pod8	0.80952	0.85250	0.63473	0.00129
Schoinoussa	Pod1a	0.52381	0.43786	0.60919	0.00161

Key: ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

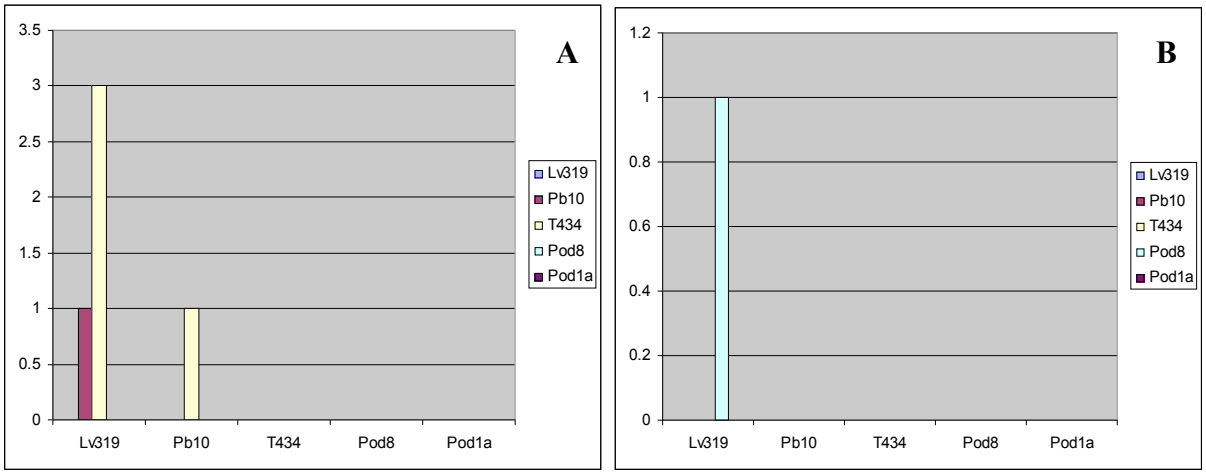


Figure F: Histograms representing the loci where linkage disequilibrium was detected after Bonferroni correction on the island of Andreas (A) and Agrilou (B).

VITA

Heather Hayden Hurston was born November 7, 1981 in Gulfport Mississippi. She received the Bachelor of Science in Biology from the University of New Orleans in 2004. She has been pursuing her Masters of Science in Biology at the University of New Orleans from 2004 to present. She has served as a research assistant at the University of Texas at Austin from October 2006 to present.